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Exposure of humans to lethal or clearly harmful levels of toxicants is straightforward to assess. Counting the number of survivors or enumerating injuries provides an accurate, repeatable method for assessing toxicant effect. However, chronic exposure to very low levels of toxicants is much more problematic. Effects to very low levels of toxicants often produces effects temporally separate from exposure and not linkable in a cause and effect relationship. Our study probed the relationship between low levels of toxicants and neurological responses. After exploratory assays of various neurotoxic chemicals, we used trimethyltin to assess neurological damage to embryos, the most sensitive stage of the life cycle. We found that we were able to detect these low levels of trimethyltin by video image analysis of neural fields using the electrochromic dye Di-4-ANEPPS. We also used analysis of retrograde transport of scrape-loaded tracer dye through neurons. We found that there was no detectable difference in the neuronal paths traced by the tracer dye.

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FOREWORD

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May 6, 1996

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Table of Contents

Figures, Tables and Pictures (micrographs)

All following experiments were performed with frog embryos. F indicates a figure, T indicates a table, and P indicates a photomicrograph.

Executive Summary

A principal concern of toxicology whether it be mammalian, vertebrate, or environmental is and will continue to be the effect of low, sub-lethal, concentrations of toxicants on organisms. One effect of low-level toxicants is on neural properties. That is, low levels of naphthalene do not kill aquatic organisms but do alter behavior. We used frog tadpoles to evaluate the effect of low toxicant levels on neurophysiology.

An existing bioassay was extended into neural and neurological analysis. FETAX (Frog Embryo Teratogenesis Assay:Xenopus) was published by Dumont in the early eighties (1) and extended and validated by Bantle, Dawson, and Fort (2). FETAX uses embryos from a fully aquatic frog, *Xenopus laevis*, as a model for vertebrate and mammalian development. The endpoints normally associated with FETAX are percent lethality, percent malformation, percent control growth, and minimum concentration to inhibit growth. To better model the mammalian embryonic environment, a Metabolic Activation System was included in the assay with promising results(3).

CHAWQ (Cell Health Assay of Water Quality) was developed in the late eighties by Blankemeyer as a cellular assay which uses a suite of cellular biomarkers to establish the mode and mechanism of action of toxicity(4). The cellular biomarkers are:

Membrane potential(5,6)
Cellular calcium (7,8)
Cell necrosis (8)
Cellular pH (8,9)
DNA quantity/environment(8,10)

CHAWQ uses *Xenopus* embryos, usually in the small cell blastula stage(11), so that the cellular biomarkers can be interpreted in terms of FETAX endpoints. Other stages of frog embryos can and have been used as well as *Daphnia*, minnows, and mammalian muscle and erythrocytes.

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Literature Review

During the course of this contract we investigated the properties and effect on embryos of several neurotoxic chemicals. A brief review of those chemicals follows:

Acrylamide

Acrylamide (2-propenamide), a benzene derivative, is a widely used industrial chemical in water treatment, construction, and oil recovery and is widely used in as a polymer in biological research. The monomer is clearly toxic to humans, with rapid skin absorption accompanied by symptoms of ataxia, numbness of limbs, fatigue, and eye and skin irritation (20).

One of the neurological modes of action is the reduction of conduction velocity. In one set of experiments, subcutaneously injected acrylamide (at 0.35 mM/kg) reduced sciatic nerve conduction velocity and subsequent paralysis of hindlimbs in six weeks in Wistar rats. Weight of soleus and plantaris muscles was reduced at this dosage level but not at the high dosage level (31).

Dose-dependent acrylamide modes of action are also suggested in human exposure although the data has been extrapolated from the usual rat models. Behavioral endpoints including grip and acoustic startle reflex were assessed under a dose vs. time paradigm. Results suggested that "behavioral endpoints showed both qualitative and quantitative changes as a result of dose rate". (27)

Mechanism of action studies employing cell lines showed that acrylamide altered the gross morphology and induced neurofilament accumulation in PC12 cells, a pheochromcytoma cell line. Acrylamide also regulated the neurofilament synthesis via the mRNA of three neurofilament subunit genes but not via NGF. Dexamethasone antagonized acrylamide induced neurofilament synthesis. These studies suggest there is a dexamethosone-sensitive step common to NGF and acrylamide mechanisms of action.(30)

These results show why acrylamide is a widely used model for rat autonomic neuropathy and may be important in development of neurophysiological biomarkers with meaningful endpoints. (Costa) The dose-dependent mechanisms apparently operating in acrylamide toxicity are probably representative of complex mechanisms underlying apparently simple modes of action. (29) and has been adopted as a standard method of producing neuropathology in rat models.

ALBIZZIA

Albizzia is probably one or more of the Julibrosides, isolated from the stem bark of the silktree, *Albizzia julibrissin*. We were unable to locate any specific references to toxicity although there is considerable interest in bio-ameliorative effects (#2).

Colchicine

Colchicine, an alkaloid from *Colchicum autumnale*, is a tool in cell biology that inhibits the doubling of chromosomes by interfering with microtubular assembly and organization during late anaphase/telophase. The same mechanism apparently operates to alter anterograde or retrograde transport in axons thus inhibiting movement of vital materials from/to the soma or cell body (20).

Cochicine is toxic to mice at doses of 3.5 mg/kg i.v. There have been attempts to use colchicine as an antineoplastic drug and as an anti-cirrhotic; these form the bulk of the literature save the use of colchicine as a molecular probe in cell biology.(20)

When rats with CCl_4 induced cirrhosis were treated with colchicine and trimethylcolchicinic acid (a colchicine derivative) significantly reversed liver disease markers suggesting that the effect on cirrhotic livers was mechanistically based. (39). Similar studies using colchicine with lactosimated serum albumin suggest that the combination was more effective than colchicine alone and that the morphological effect of the colchicine was to reduce fibrotic activity.(43)

Glutamate

Glutamate has neurotoxic effects characteristic of the excitatory amino acids (EAA) and a wide range of other effects including altering the metabolism of ischemic cardiac cells and antiproliferative activities in rheumatoid cell culture models (see 44-49).

Glycine

Glycine, as is glutamate, evokes significant interest as a member of the EAA group, a set of amino acids that have interesting properties, generally associated with agonist or antagonist properties of the NMDA (N-methyl-D-aspartate) effects. Current interest is in motor activity in relationship to PCP (53)

and the glycine site in the NMDA complex (54).

Glycoalkaloids

Solanaceous plants, including crops such as potatoes, tomatoes, and eggplant as well as the toxic weeds black nightshade and jimsonweed, and tobacco, synthesize secondary plant metabolites during growth and post-harvest storage and processing. Glycoalkaloids are secondary plant metabolites in potatoes whereas the primary glycoalkaloid in tomatoes is tomatine (1). In commercial potato cultivars, the primary glycoalkaloids are α-chaconine and α-solanine, present in approximately equal amounts (2). These glycoalkaloids have similar structures except for the differences in carbohydrate side chains. For example, α-chaconine has a glucose-rhamnose-rhamnose side chain whereas α-solanine has a galactose-glucose-rhamnose side chain. Glycoalkaloids are present in highest concentrations when the potato or tomato is green, or damaged, or stored potatoes (3). That is, a section of a potato that is green will have a higher concentration of glycoalkaloids than a nearby white portion of the potato. Mishandling or poor storage increases the concentration of glycoalkaloids. We can infer that the glycoalkaloids evolved as a plant defense mechanism against herbivores and are probably toxic (4).

TOXIC EFFECTS OF GLYCOALKALOIDS

Potato glycoalkaloids are implicated in human toxicity. A surprisingly low level of potato glycoalkaloids, ca. 5 mg/kg, is lethal to humans(5). α-Chaconine is linked to spinal defects in mammalian embryos such as *spina bifida* (5). *Spina bifida* occurs at a frequency of 5 per 1000 live births(6). Analysis of miscarried pregnancies shows that the frequency of *spina bifida* per conceptions is ca. 20 per 1000 (6). Consumption of potatoes by humans results in significant serum levels of glycoalkaloids and glycoalkaloid by-products (6,7). Of the many animal models exposed to the glycoalkaloids, hamsters are the most sensitive to glycoalkaloids and mimic suspected human sensitivity to glycoalkaloids (8). While mammals are clearly affected by glycoalkaloid toxicity, many studies have employed non-mammalian models to increase sample size and ease of experimental manipulation. For example, frog embryos are affected in two clear modes: [1] high concentrations of glycoalkaloids (2 mg/L) are embryo-lethal; [2] lower concentrations (ca. 0.1 mg/L) are teratogenic (2). Some of the malformations observed upon exposure to α-chaconine are anencephaly, lack of neural tube closure, or other spinal defect, pathologies very similar to those demonstrated in glycoalkaloid toxicity to mammals. Since glycoalkaloids produce similar spinal failures in frogs as in

humans, frogs are a reasonable model for studying interactions of toxicants with development.

BENEFICIAL EFFECTS OF GLYCOALKALOIDS

Besides the well-established toxicity of the glycoalkaloids to vertebrates, glycoalkaloids are also quite toxic to fungal growth (). Solasodine glycosides have been suggested as anti-cancer agents ().

EFFECT OF FOLIC ACID ON NEURAL TUBE PATHOLOGY

Folic acid has been linked to the prevention of human spinal defects by strong epidemiological evidence (12). In the membrane potential assay with frog embryos, folic acid reduces the toxicity of the glycoalkaloids when membrane potential is used to measure toxicity (see Preliminary Data). Data linking glycoalkaloids with membrane potential changes suggests that the mechanism of action is a specific effect on cell membranes.

KAINIC ACID (KAINATE)

Kainic acid is normally associated with epileptic seizures, occurring after administration to mammals. When the brains of these animals or when kainic acid is directly injected or otherwise positioned near brain tissue, a lesion occurs with a clear cause and effect relationship. It is usually associated with the class of excitatory amino acids or EAA's. Typical of EAA associated activity is N-methyl-D-aspartate or NMDA (see 56-60).

MIMOSINE

Mimosine is a toxic non-protein amino acid that is a major constituent of the tropical legumes *Leucaena* and *Mimosa*. Mimosine causes acute and chronic toxicosis in livestock ingesting those legumes. In cell culture trials, mimosine blocked DNA synthesis when applied at pharmacological levels (0.4 mM)(62). At least in this system mimosine has a mechanism of action consistent with iron chelation since the DNA synthesis inhibition was reversed by addition of excess iron sulfate.

NAPHTHALENE

We have worked extensively with naphthalene in our lab, using it as a model "oil field" pollutant to test the response of organisms, including frogs and daphnia, to field doses of naphthalene (13). Naphthalene is an oil byproduct that has many industrial uses as well as being an insecticide.

TRIMETHYLTIN

Trimethyl tin is an organometal chemical that produces selective lesions in hippocampal pyramidal cells in both neonate and learning dysfunction in adult rats (67,68).

VINBLASTINE

Vinblastine, a vinca alkaloid, is an antineoplastic drug that strongly affects the assembly of microtubules and thus alters neural anterograde transport and other cellular, microtubule dependent, processes (20). The antineoplastic activity of vinblastine is probably due to a reduction in blood flow to the tumor (73).

HYPOTHESIS

Hypothesis: Measurement of neurological endpoints will extend FETAX assay and correlate effects of sub-lethal concentrations of toxicants with measures of neurotoxicity.

Technical Objectives:

1 To determine whether the test chemicals affect neural development and neural properties, we will qualitatively and quantitatively measure tadpole neuron location as well as neuronal properties.

2 To establish the relevance of the neurological endpoints, we will use a conventional bioassay and a cellular bioassay in conjunction with the neurological assays. Conjointly assaying the chemicals permits direct comparison of results using matched pair statistics.

3 Accomplishment of these Technical Objectives will result in experimental paradigms, protocols and biological endpoints for integrated bioassessment.

METHODS

FETAX DESCRIPTION

FETAX (Frog Embryo Teratogenesis Assay: *Xenopus*) uses South African clawed frog embryos in assessing survival, growth, and malformation as endpoints in a four-day assay (2). Use of the embryonic stage of the organism probably exposes the most sensitive stage of the life-cycle to the toxicant. Use of the multiple endpoints enables detection of multiple effects of toxicants (2). FETAX has been evaluated with over sixty chemicals, is established as an ASTM standard (2), and is now undergoing inter-lab validation. The copious amount of data existing for FETAX makes validation of the membrane potential and other CHAWQ (Cell Health Assay of Water Quality) biomarkers realistic since there is a 96 hour FETAX response available for most of the chemicals. Dr. Jack Bantle, who is responsible for standardizing FETAX and responsible for its worldwide reputation, is the co-P.I. on this proposal. The CHAWQ assay (4,8), using a suite of cellular biomarkers, developed to assess the cellular mode and mechanism of toxicity, uses the same breeding and embryo selection protocol as the FETAX assay.

CHAWQ DESCRIPTION

CHAWQ (Cell Health Assay of Water Quality), developed by me over the last four years, uses blastula stage embryos is to rapidly assess the quality of water and to determine the mode and mechanism of action of toxicants. The rationale behind CHAWQ is that long-term deleterious effects on cells exposed to water will be reflected in short-term alterations of cellular biomarkers. CHAWQ uses 30 embryos per replicate and takes about thirty minutes to perform. CHAWQ evaluates principal indicators of cellular health by using non-invasive optical transducers of cellular biomarkers (8). The CHAWQ assay has been used to evaluate the toxicity of potato glycoalkaloids (4) and been calibrated for membrane potential response using valinomycin and gramicidin as known modifiers of membrane permeability (5). The calibration study showed that an increase in dye fluorescence is correlated with a depolarization of the negative cell membrane potential towards zero. An ASTM monograph as well as other publications (4,5,8) delineates the instrumentation, biomarkers, and shows a CHAWQ can be used to discriminate mode and mechanism of action. Lastly, a patent application and is in review for the use of the technique on *Daphnia* as a rapid replacement for the four and seven-day *Daphnia* survival and reproduction assays for water quality.

The extension of the bioassays into neural analysis was

suggested by the studies of Kandel and collaborators on *Aplysia*. Kandel (see 18 for instance) showed, in an impressive series of experiments, that conditioning of the siphon withdrawal reflex in Aplysia was associated with a demonstrable change in the dendritic structure surrounding the identified interneurons. The simplicity of the Aplysia neural network aided in the analysis; however similar techniques can be applied to vertebrates with respect for the limitations imposed by smaller neuronal size and complex neural architecture of vertebrates.

We employed naive and pre-exposed tadpoles in our analysis of behavior. We will use the tadpoles surviving the FETAX and CHAWQ assays as experimental subjects for the neurological assays. We will use the control tadpoles from FETAX and CHAWQ as naive tadpoles for our neurological studies. We will use exposed tadpoles as pre-exposed tadpoles in the neurological studies.

D.4.1 CHAWQ INSTRUMENT

Figure 1 is a schematic layout of the instrumental portion of the CHAWQ instrument. The light from a xenon short-arc lamp (not shown) enters two monochromators (MONO1 and MONO2). The monochromators have mechanically operated slits and electro-mechanical monochromator drives (not shown). The light outputs of the monochromators are combined by an optical chopper into a single beam containing the two wavelengths from the monochromators.

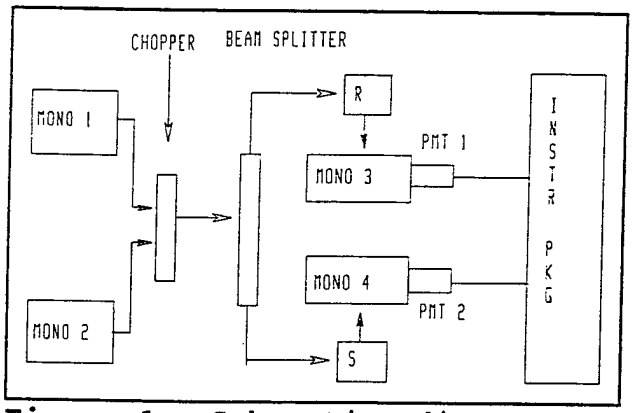


Figure 1. Schematic diagram of CHAWQ instrumentation

The combined beam is split into two beams by a beam splitter (BEAM SPLITTER). One beam goes through a sample chamber (S); the other passes through the reference chamber (R). The sample chamber consists of 3 mm quartz microcuvette mounted inside a baffled aluminum sample box (see Figure 2 for details). Inside the sample chamber (the microcuvette) are the test embryos. The combined light beam is used to obtain fluorescence data from both the sample and reference organisms (eg. frog embryos). The beam excites the electrochromic dye Di-4-ANEPPS which has been pre-loaded into the embryos. Di-4-ANEPPS fluoresces proportionally to the membrane potential. The fluorescent emission is measured by counting the photons passing through the emission monochromators (MONO3 and MONO4). The counting is accomplished by photon-counting the output from photomultiplier tubes (PMT) and transferring the count rate to an instrumentation package.

Figure 2 is a detailed representation of the sample chamber portion of CHAWQ. That is, the boxes labeled R and S in Figure 1 are drawn in Figure 2 in detail. In Figure 2, light enters the sample chamber from the left of the figure and is focused onto the central box which contains a 3 mm quartz microcuvette. Baffles (drawn but not labelled) prevent the light from being inadvertently scattered into the emission monochromator. The light entering the microcuvette excites the embryos containing electrochromic dye Di-4-ANEPPS (or other fluorescent dye). The emission from the dye-loaded embryos exits at a 90 ° angle to the excitation light. The emission from the embryos is captured and focused onto a monochromator (not shown) which is attached to the exit port.

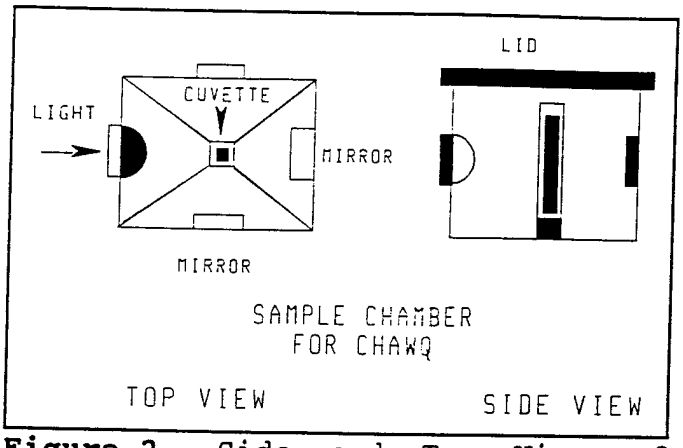


Figure 2. Side and Top View of Sample Chamber

Figure 3 describes the loading process used to place the embryos in the 3 mm quartz cuvette in Figure 2. The blastula-stage embryos are loaded into the cuvette by drawing the embryos into a widened, fire-polished pipette. The embryos are then carefully dropped out of the pipette into the 3 mm cuvette, previously filled with FETAX solution (16). A separate pipette, attached to a vacuum line, is used to draw off excess FETAX solution. Since the embryos have less than neutral buoyant density in FETAX solution, the embryos slowly sink to the bottom and stack vertically. The entire process of loading and reading the sample with the CHAWQ instrument takes less than two minutes. Since the exposure time in the cuvette is so short, no stirring or temperature control has been required.

VIDEO IMAGE ANALYSIS BIOASSAY

To answer some of the questions raised in this proposal, we will morphologically analyze the activity and location of neural

pathways in the tadpoles. The absence of pigment in the albino *Xenopus* tadpole makes the tadpole an excellent experimental subject for this assay. We will, using the same Di-4-ANEPPS dye as used in the CHAWQ assay, identify single neurons and bundles by differences in membrane potential between the neurons and the surrounding tissue. The neurons have a resting potential of ca. -70 milliVolts while the tissue has a typical membrane potential of -30 millivolts. In preliminary experiments we have been able to analyze moto-neurons against the background of muscle.

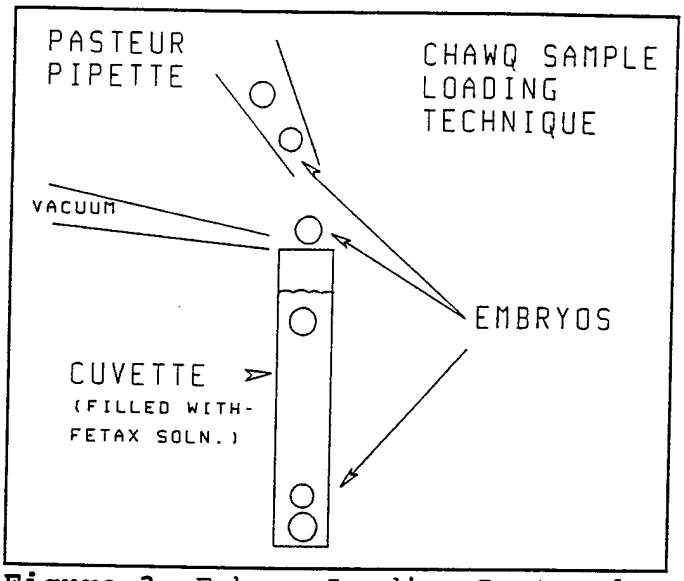
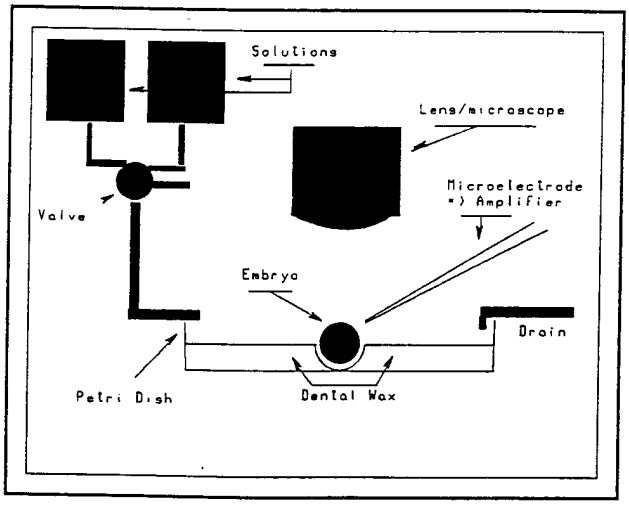


Figure 3. Embryo Loading Protocol

We will use the dye to assess location of particular neurons. We expect to identify vagal, brachial, and femoral bundles. We will assess the membrane potential, using the Di-4-ANEPPS dye, firing rate, and anatomical location using the image processor setup described for the avoidance-preference and swimming bioassay. We will use a multichannel image intensifier to acquire the faint images from the neurons.

NEURONAL PATTERN ANALYSIS

We will evaluate the neural pathways using neuronal tracing techniques. There are a variety of neuronal tracers available commercially. These include: Lucifer Yellow, Cascade Blue, and the CellTracker series from Molecular Probes. We plan to use these dyes in anterograde and retrograde tracing. These dyes are injected into the cells by the following means:



Iontophoretic injection or pressure injection- Dye is loaded into a micropipette and injected into a particular neuron. In vertebrates the problem is in identifying the neuron prior to injection. Once the dye is injected, the dye diffuses through the

axon and is localized through fluorescence image analysis.

Ablation of a neural field or scrape loading relies on a broad-brush loading of neurons by severing the neurons at a particular point, then following the course of the dye diffusion by fluorescence analysis.

DYE/MICROELECTRODE DESCRIPTION

Microelectrode experiments were carried out using a setup schematicized in Figure 4 as modified for this project. The microelectrode is mounted in a mechanical micromanipulator mounted on the microscope stage. The microelectrode is "pulled" from borosilicate glass using an ISA or Narashige horizontal microelectrode puller. These pullers usually make electrodes in the 10 megohm range with steep tapers and concentric tips. The electrodes are backfilled with KCl solution and tip-filled through capillary action from the fluorogold solution.

Embryos were placed in dishes Of FETAX and allowed to grow for 48+ hours. An embryo was chosen from the group and anesthetized. An injection of Fluro-gold was given into the eye of the embryo. The amount of 73.6 nl was measured by the Nanoject pump. The embryo was then placed into a dish of FETAX.

Embryos developed for another 96+ hours. At this point, they were anesthetized and formalined. The specimens were prepared for sectioning by placing them on a metal block already frozen in liquid nitrogen) and dropping water around La provide an adhesion to the surface. - The block was then Placed into the liquid nitrogen until the water Was completely frozen.' :The Cryostat was cooled to -20 degrees C. The block was then placed into the holder and 20 micron sections were obtained. They were placed sequentially onto a slide for viewing. The sections were viewed with a fluorescence microscope. A blue/green 420K blocking filter was used. Photos were taken with a 40X objective and 5X photo eyepiece.

ANIMAL CARE

PROTOCOL

Adult albino *Xenopus laevis* pairs were kept in aquaria and periodically mated to produce fertilized eggs. These embryos are the experimental subjects for the proposed research. All embryos are terminated at the end of the experiments (typically 96 hours after fertilization).

Adult pairs are bred about every six weeks. HCG (human chorionic gonadotropin) is injected into the *Xenopus laevis* female and male

about 12 hours prior to the experiment. About six hours after HCG injection, the adults will perform amplexus. About 500 embryos are obtained from each mating. The embryos are de-jellied and sorted into viable and non-viable embryos. The viable embryos are then placed into petri dishes in FETAX solution. At the appropriate time, depending on the experiment, the embryos are sorted into groups of 20 and labeled as control and experimental. Chemicals and dyes are introduced into the various dishes as the particular protocol requires. Typically several groups will be designated as control and graded concentrations of toxicant will be added to multiple dishes using three groups for each concentration of chemical. At about four hours after fertilization, the assay is performed on the embryos (CHAWQ assay - see Experimental Design and Methods). The embryos were euthanized after eight hours or are maintained until 96 hours so that the FETAX measurements of length, malformation, and viability can be collected on the groups of embryos(control and experimental). After 96 hours all embryos are euthanized. Adults are kept as long as possible for breeding stock and are never used in experiments. When the adults become debilitated, diseased, or dysfunctional, they are euthanized. As far as we can understand there is no pain, distress, or discomfort to the experimental subjects (four hour embryos). Since the embryos do not have developed nervous systems at this stage we believe that there is no distress from the dyes, toxic chemicals, or measurement techniques.

Euthanasia

Embryos were terminated at 96 hours by anesthetizing embryos in 3-amino benzoic acid ethyl ester (CAS # 886-86-2) and euthanizing the embryos in a formalin solution. Adults are kept for as many breedings as possible. Diseased or dysfunctional adult frogs are euthanized also with 3-amino benzoic acid and formalin solution. This method of euthanasia is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association Panel on Euthanasia JAVMA 188 (3) : 252-268 (1986).

STATEMENT OF WORK/SUMMARY OF RESULTS

The Statement of Work was modified during the course of the study to accommodate the new exciting results uncovered by the research. We found that the excitatory amino acids (EAA) had no effect on frog embryos and thus were unsuitable candidates for further testing on the whole animal level. Then, in consultation with the CO we altered the research to include testing of trimethyl tin (TMT). TMT proved to be a potent chemical in our assay. Use of TMT permitted us to achieve the original goals of the research without major alteration in research plan or methodology. We analyzed the effects of TMT with FETAX, CHAWQ, video image analysis, and neuronal tracing. We found clear evidence that the presence of TMT causes localized changes in the membrane potential of regions of the tadpoles brain and found that the pathway of traced neurons showed little or no alteration when developing embryos were exposed to TMT.

ACRONYM/SYMBOL DEFINITION

CHAWQ Cell Health Assay of Water Quality. A cellular assay capturing cellular signals leading to toxicity.

FETAX Frog Embryo Teratogenesis Assay: Xenopus. A 96 hour bioassay evaluating mortality and malformation in frog embryos.

EC50 (also ED50) Concentration or dose of toxicant producing 50 % effect.

LC50 (also LD50) Concentration or dose producing 50 % mortality in a test sample.

NOEC No observable effect concentration. Concentration of toxic chemical statistically inferred to have no significant effect on organism.

LOEC Lowest observable effect concentration. Lowest concentration of toxic chemical statistically inferred to have a significant effect on organism.

BIOASSAY An assay of toxicity which depends on biological responses as compared to chemical responses.

BIOMARKER Property or characteristic of biological systems that is uses as an indicator or the health of the organism.

IMAGE ANALYSIS Use of computers and computer software to evaluate and interpret digitized images.

RESULTS

At the commencement of the contract, we performed the preliminary analysis of response with the specified chemicals and additional chemicals suggested by the contract officer. We have completed preliminary analysis of the results and submit the results for your consideration. We used the following chemicals and performed developmental, growth, and cellular response tests on frog embryos using these chemicals:

α-chaconine
kainic acid
naphthalene
colchicine

Data from survival experiments (in tables) and fluorescence analysis of membrane potential (bar graphs) follow this page. Following is a synopsis of the results obtained.

As outlined in the proposal narrative, we initially used kainic acid (KA), colchicine, and variations on kainic acid to induce central nervous system pathologies. Unfortunately, the embryos neglected to cooperate. They did not respond to any concentration of KA or colchicine during the 96 hour FETAX assay or CHAWQ assay (three trials each). We detected no significant differences in survivability, growth, or behavior after high doses of KA, colchicine, and some of the KA alternatives. I realize that KA and like compounds do not cross the blood-brain barrier and are routinely iontophoretically injected. However I really expected some effect especially with doses 10x the LC50 in rodents or frogs. We did get a nice positive FETAX response to vinblastine; we are repeating this experiment. The non-response to KA and colchicine caused us to change our experimental timetable. Below is a commentary on the specific experiments.

Figure 5 is an experiment illustrating the fluorescence response from the positive control experiment (labelled PC) on all subsequent experiments. This concentration of the potato glycoalkaloid, α-chaconine, produces a large increase in fluorescence and was thus used to provide a positive control parameter for subsequent tests. α-Chaconine, at 50 mg/L, is 100 % lethal at 24 hours of exposure.

Table 1 is a survival and malformation experiment using naphthalene exposures for 96 h. Note that there was no difference in survival between the negative control and the various exposures to naphthalene. This result is consistent with previous exposure experiments.

Table 2 represents one of the exposure experiments to kainic

acid or kainate. There was no significant difference between negative control and experimental data points. The positive control, the 5 mg/L of α-chaconine, was lethal top 100 % of the embryos by the end of the second day. Table 3 and Table 4 are the results from additional trials of kainic acid. As occurred in the first trial (Table 2), there was no significant difference between negative control survival and the experimental concentrations of kainic acid.

We next used various concentrations of colchicine in survival and malformation experiments. Tables 5, 6, and 7 represent results from experiments determining the survival and malformation from concentrations of colchicine. In one trial, represented in Table 7, we saw some mortality but only at unrealistically high concentrations of colchicine.

Figure 6 represents the fluorescence response of embryos exposed to naphthalene and negative and positive controls. In this experiment there was no significant difference among the response to the negative control and the concentrations of naphthalene. Figure 7 represents an experiment with embryos that were refractile to the positive control. In subsequent experiments we discarded trials such as this one. It is included here to illustrate the acceptability of trial data.

Figure 8 represents the results of experiments wherein kainic acid was used to in a fluorescence trial using the CHAWQ protocol . Note the marked increase in fluorescence due to the positive control and that the various concentrations of kainic acid produced a fluorescence response not significantly different from the negative control.

Colchicine was used to test the response of the embryos in a trial depicted in Figure 9. In this experiment the PC produced a marked increase in fluorescence whereas the negative control results were indistinguishable from the various concentrations of colchicine.

After the disappointing results and after discussion with the contract officer, we tested compounds that had neurotoxic modes of action. Those toxic compounds included: naphthalene, α-chaconine, colchicine, methotrexate, and mimosine. Naphthalene has general neurotoxic effects unfortunately not limited to the CNS. α-chaconine has neurotoxic effects at low concentrations that are mostly restricted to the CNS. Methotrexate has developmentally neurotoxic effects on frog embryos. Mimosine has no effect on embryos survivability or growth but produce amazing behavioral changes. The tadpoles show strange swimming patterns, often swimming in circles or in a few cases always swimming upside down.

After we completed the preliminary testing, we initiated the testing of responses of the frog embryos by exposing the embryos during temporal windows of toxicant exposure and analyzing the membrane potential response by fluorescence video image processing and photographic means. We initiated retrograde neural tracing by exposing embryos to fluoro-gold and tracing neural pathways. We setup and perfected our cryostat and image analysis software. We included completed testing, not furnished with the last report, from the first quarter of the Contract and preliminary results from the second quarter of the contract. We used the following chemicals and performed developmental, growth, and cellular response tests on frog embryos using the below-mentioned chemicals:

- **α-chaconine**
- **vinblastine**
- **mimosine**
- **glutamate**
- **glycine**
- **homocysteic acid**
- **albizia**

Results from survival experiments (in tables) and fluorescence analysis of membrane potential (graphs) follow this preliminary page.

As previously described (above), we initially used kainic acid (KA), colchicine, and variations on kainic acid to induce central nervous system pathologies. Unfortunately, the embryos neglected to cooperate. They did not respond to any concentration of KA or colchicine during the 96 hour FETAX assay or CHAWQ assay (three trials each). We detected no significant differences in survivability, growth, or behavior after high doses of KA, colchicine, and some of the KA alternatives. These results follow. Use of some plant glycoalkaloids showed interesting toxicity (mimosine) or behavioral responses (albizia)

The non-response to KA and colchicine also caused us to change our experimental timetable and to use α-chaconine as a chemical to invoke neuropathology. Using timed exposure of embryos to low doses of α-chaconine, we were able to assess the effect on embryonic membrane potential on a regional basis and sometimes on an organ by organ basis. We include a few of the many images we have gathered. These images are captured by video image processing using hardware and software and by photographic means.

Table 8 represents the results obtained from vinblastine exposures of frog embryos. In this experiment high doses of vinblastine, 5 and 10 mg/L produced a slight effect of survival of the embryos on day 4 (96h). Significantly all of the dead embryos showed significant malformation. Table 9 presents the results of a second trial with a larger range of concentrations. Again the higher concentrations produced death and malformation. Figure 10 graphically portrays the fluorescence results from a CHAWQ assay. In this figure a slight decrease in fluorescence (meaning a hyperpolarization of the membrane potential) was observed at concentrations near that required to produce noticeable malformation/death in survival experiments. Figure 11 is a trial using higher ranges of vinblastine. Fig 12 is a low dose experiment using α-chaconine.

Figure 13 is a trial using the CHAWQ protocol of an interesting alkaloid, mimosine. We see from this figure that there is a clear membrane potential effect that is biphasic. That is low concentrations of mimosine depolarize the membrane whereas high concentrations (>~15 mg/L) hyperpolarize the membrane. Table 10 represents a trial using mimosine on the 96 h survival assay. Unfortunately we had poor survival in the controls so the data is suspect. Unfortunately we were unable to obtain more mimosine to continue our study of this interesting alkaloid.

Table 11 represents experiments using the rare alkaloid abizzia on survival and malformation of embryos. Albizzia had no effect on survival or malformation but had clear effects on behavior. Upside down swimming and circling behaviors were observed in these 96h tadpoles.

Figure 14 represents the results of experiments wherein the EAA glycine was used in CHAWQ fluorescence assays. Except for some unexplained hyperpolarization at low concentrations, there was little effect on the membrane potential. Figure 15 for glutamate, another EAA, and Figure 16 for homocysteic acid.

Table 12 is the order of battle for the series of

photographs following the table. These photographs identify and report the effect of application of α-chaconine during the later developmental stages. We used the CHAWQ protocol but did not examine the embryos with fluorescence until the latter days of the trial. Subsequent experiments applied the dose of α-chaconine later (after 24h).

This next section is a pictorial view of glycoalkaloid toxicity. Embryos were expose to Di-8-ANEPPS and low concentrations of α-chaconine.

Based on the results from the first half of the contract we employed additional chemicals suggested by the contract officer at no additional cost or expense to the Army. Also, based on comments from the Contract Officer, we delayed microinjection of KA and similar compounds into the developing embryos. We used other neurotoxicants suggested by the contract officer, eg. acrylamide, trimethyltin.

During the third quarter of the contract, we initiated the testing of responses of the frog embryos by exposing the embryos during temporal windows of toxicant exposure and analyzing the membrane potential response by fluorescence video image processing and photographic means. We concentrated on analyzing the effect of trimethyltin on the neural pathways of frog embryos. As can be viewed in our data, these efforts met with success. We were able to demonstrate changes in membrane potential patterns when trimethyl tin embryos were compared to controls. We used the following chemicals and performed developmental, growth, and cellular response tests on frog embryos using the below-mentioned chemicals:

- **trimethlytin**
- **TCAOB & TCAB**
- **acrylamide**

Results from survival experiments (in tables) and fluorescence analysis of membrane potential (graphs and pictures) follow.

Table 13 describes an experiment using an environmental toxicant TCAOA and TCAOB. These were generously provided by Dr. Jim Catallo. TCAO is a food derived toxic chemical with suspected neural properties (i.e. behavioral aberrations). Unfortunately the chemical is stage specific killing and malforming the embryos at the end of day 1 (24+ h). Unfortunately we had to use a very high concentration of DMSO so in this experiment we were unable to conclude that the TCAO was toxic. When we used 1 % DMSO to dissolve the TCAO, we found that the chemical was toxic but only after the embryos had reached the tadpole stage. We are still

not sure what the mechanism of toxicity is for this chemical. The 1% results are presented in Tables 16 and 24.

Acrylamide had a mild toxic effect even at high concentrations. These results are presented in Table 14 and Table 17. Figure 17 suggests there is some effect on the embryos, just not a lethal one since the effect of acrylamide is to hyperpolarize the membrane potential (lower fluorescence).

Table 15 presents a quality control experiment with our positive control. We run these periodically to insure our system is still producing the same responses as earlier trials.

Trimethyltin (TMT) was tested first at high concentrations. In Table 18, we note that all of the embryos were dead after day 2 in 10 mg/L TMT. At lower concentrations (Tables 19 - 23) all of the embryos were deformed and dead while there was almost 100% survival in the negative controls. From these experiments we infer that the Ec50 for malformation is near 0,5 mg/L while the LC50 is near 7 mg/L.

Figures 18 and 19 represent a fluorescence response to trimethyltin. Note the hyperpolarization at low doses.

During the fourth quarter of the contract, we initiated the testing of embryos by analyzing the response of neural tissues to trimethyltin using to evaluation paradigms, video image analysis of living tadpoles using voltage sensitive dye and tracing of the injected dye Fluorogold through retrograde transport. In the case of the voltage sensitive dye clear changes in intensity and pattern were noted and analyzed to the level of the available equipment. We noted that certain regions of the tadpole brain were selectively illuminated upon exposure to TMT. These responses are photographically presented in the pages following this narrative. Brighter regions are interpreted as depolarization of the cell membranes in the region. The last set of pictures is a tracing of the retrograde transport of FluoroGold in a control embryos. The sections were cut on a cryostat and then photographed with UV excitation to limit background fluorescence. The tadpoles exposed to TMT showed no significant difference in pattern.

DESCRIPTION OF PHOTOMICROGRAPHS

FLUORESCENCE OF ALBINO EMBRYOS EXPOSED TO LOW CONCENTRATIONS α-CHACONINE

Photomicrograph 1: 24+ hour old embryo incubated in FETAX solution for 24 hours then di-8-Anepps for 30 min. Exposure time is 5 sec. Negative control for photomicrographs 1-6.

Photomicrograph 2: 24+ hour old embryo incubated in 0.50 mg/L α-chaconine for 24 hours then di-8-Anepps for 30 min. Exposure time is 5 sec.

Photomicrograph 3: 24+ hour old embryo incubated in 1.00 mg/L α-chaconine for 24 hours then di-8-Anepps for 30 min. Exposure time is 5 sec.

Photomicrograph 4: 24+ hour old embryo incubated in 2.50 mg/L α-chaconine for 24 hours then di-8-Anepps for 30 min. Exposure time is 5 sec.

Photomicrograph 5: 24+ hour old embryo incubated in 5.00 mg/L α-chaconine for 24 hours then di-8-Anepps for 30 min. Exposure time is 5 sec.

Photomicrograph 6: 24+ hour old embryo incubated in 10.0 mg/L α-chaconine for 24 hours then di-8-Anepps for 30 min. Exposure time is 5 sec.

Photomicrograph 7: 72+ hour old embryo incubated in FETAX solution for 48 hours then 0.25 mg/L α-chaconine for 24 hours and di-8-Anepps for 30 min. Exposure time is 5 sec. Can be used as negative control for photomicrographs 7-12.

Photomicrograph 8: 72+ hour old embryo incubated in FETAX solution for 48 hours then 0.50 mg/L α-chaconine for 24 hours and di-8-Anepps for 30 min. Exposure time is 5 sec.

Photomicrograph 9: 72+ hour old embryo incubated in FETAX solution for 48 hours then 1.00 mg/L α-chaconine for 24 hours and di-8-Anepps for 30 min. Exposure time is 5 sec.

Photomicrograph 10: 72+ hour old embryo incubated in FETAX solution for 48 hours then 2.50 mg/L α-chaconine for 24 hours and di-8-Anepps for 30 min. Exposure time is 5 sec.

Photomicrograph 11: 72+ hour old embryo incubated in FETAX solution for 48 hours then 5.00 mg/L α-chaconine for 24 hours and di-8-Anepps for 30 min. Exposure time is 5 sec.

Photomicrograph 12: 72+ hour old embryo incubated in FETAX

solution for 48 hours then 10.0 mg/L α-chaconine for 24 hours and di-8-Anepps for 30 min. Exposure time is 5 sec.

FLUORESCENCE OF ALBINO EMBRYOS EXPOSED TO LOW CONCENTRATIONS OF TRIMETYLTIN

Photomicrograph 13: 24+ hour old embryo incubated in FETAX solution for 24 hours then in di-4-Anepps for 4 hours. Exposure time is 1 sec. Negative control for pictures 13-18.

Photomicrograph 14: 24+ hour old embryo incubated in FETAX solution for 24 hours then in 0.10 mg/L trimethyltin and di-4-Anepps for 4 hours. Exposure time is 1 sec.

Photomicrograph 15: 24+ hour old embryo incubated in FETAX solution for 24 hours then in 0.25 mg/L trimethyltin and di-4-Anepps for 4 hours. Exposure time is 1 sec.

Photomicrograph 16: 24+ hour old embryo incubated in FETAX solution for 24 hours then in 0.50 mg/L trimethyltin and di-4-Anepps for 4 hours. Exposure time is 1 sec.

Photomicrograph 17: 24+ hour old embryo incubated in FETAX solution for 24 hours then in 1.00 mg/L trimethyltin and di-4-Anepps for 4 hours. Exposure time is 1 sec.

Photomicrograph 18: 24+ hour old embryo incubated in FETAX for 27 hours and di-4-Anepps for 2.5 hours. Photo is with 5X objective and exposed for 1 sec. Negative control for photomicrographs 18-22.

Photomicrograph 19: 24+ hour old embryo incubated in 0.10 mg/L trimethyltin for 27 hours and di-4-Anepps for 2.5 hours. Photo is with 5X objective and exposed for 1 sec.

Photomicrograph 20: 24+ hour old embryo incubated in 0.25 mg/L trimethyltin for 27 hours and di-4-Anepps for 2.5 hours. Photo is with 5X objective and exposed for 1 sec.

Photomicrograph 21: 24+ hour old embryo incubated in 0.50 mg/L trimethyltin for 27 hours and di-4-Anepps for 2.5 hours. Photo is with 5X objective and exposed for 1 sec.

Photomicrograph 22: 24+ hour old embryo incubated in 1.00 mg/L trimethyltin for 27 hours and di-4-Anepps for 2.5 hours. Photo is with 5X objective and exposed for 1 sec.

Photomicrograph 23: Section 1 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure

time was 30 seconds.

Photomicrograph 24: Section 3 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 25: Section 4 and 5 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 26: Section 5 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 27: Section 11 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 28: Section 12 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 29: Section 13 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 30: Section 16 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 31: Section 20 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 32: Section 22 of negative control injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 33: Section 1 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30

seconds.

Photomicrograph 34: Section 3 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 35: Section 3 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 46: Section 4 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 37: Section 7 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 38: Section 8 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 39: Section 8 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 40: Section 13 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 41: Section 15 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 42: Section 15 of albino embryo incubated in 0.10

mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 43: Section 18 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 44: Section 27 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 45: Section 30 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 46: Section 33 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 47: Section 34 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 48: Section 35 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 49: Section 36 of albino embryo incubated in 0.10 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 50: Section 3 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30

seconds.

Photomicrograph 51: Section 4 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 52: Section 7 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 53: Section 8 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 54: Section 9 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 55: Section 10 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 56: Section 11 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 57: Section 12 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 58: Section 12 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 59: Section 12 of albino embryo incubated in 0.25

mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 60: Section 12 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 61: Section 14 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 62: Section 15 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 63: Section 16 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 64: Section 17 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 65: Section 18 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 66: Section 19 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 67: Section 19 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30

seconds.

Photomicrograph 68: Section 22 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 69: Section 23 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 70: Section 24 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 71: Section 24 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 72: Section 26 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 73: Section 27 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 74: Section 29 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 75: Section 30 of albino embryo incubated in 0.25 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 76: Section 35 of albino embryo incubated in 0.25

mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 77: Section 3 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 78: Section 3 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 79: Section 5 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 80: Section 6 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 81: Section 7 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 82: Section 7 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 83: Section 8 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 84: Section 10 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30

seconds.

Photomicrograph 85: Section 11 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 86: Section 13 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 87: Section 13 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 88: Section 13 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 89: Section 14 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 90: Section 15 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 91: Section 21 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

Photomicrograph 92: Section 29 of albino embryo incubated in 0.50 mg/L trimethyltin for 48+ hours and then injected with Fluro-Gold into the eye. Both a light micrograph and fluorescence micrograph were combined to show the area of tracing. Exposure time was 30 seconds.

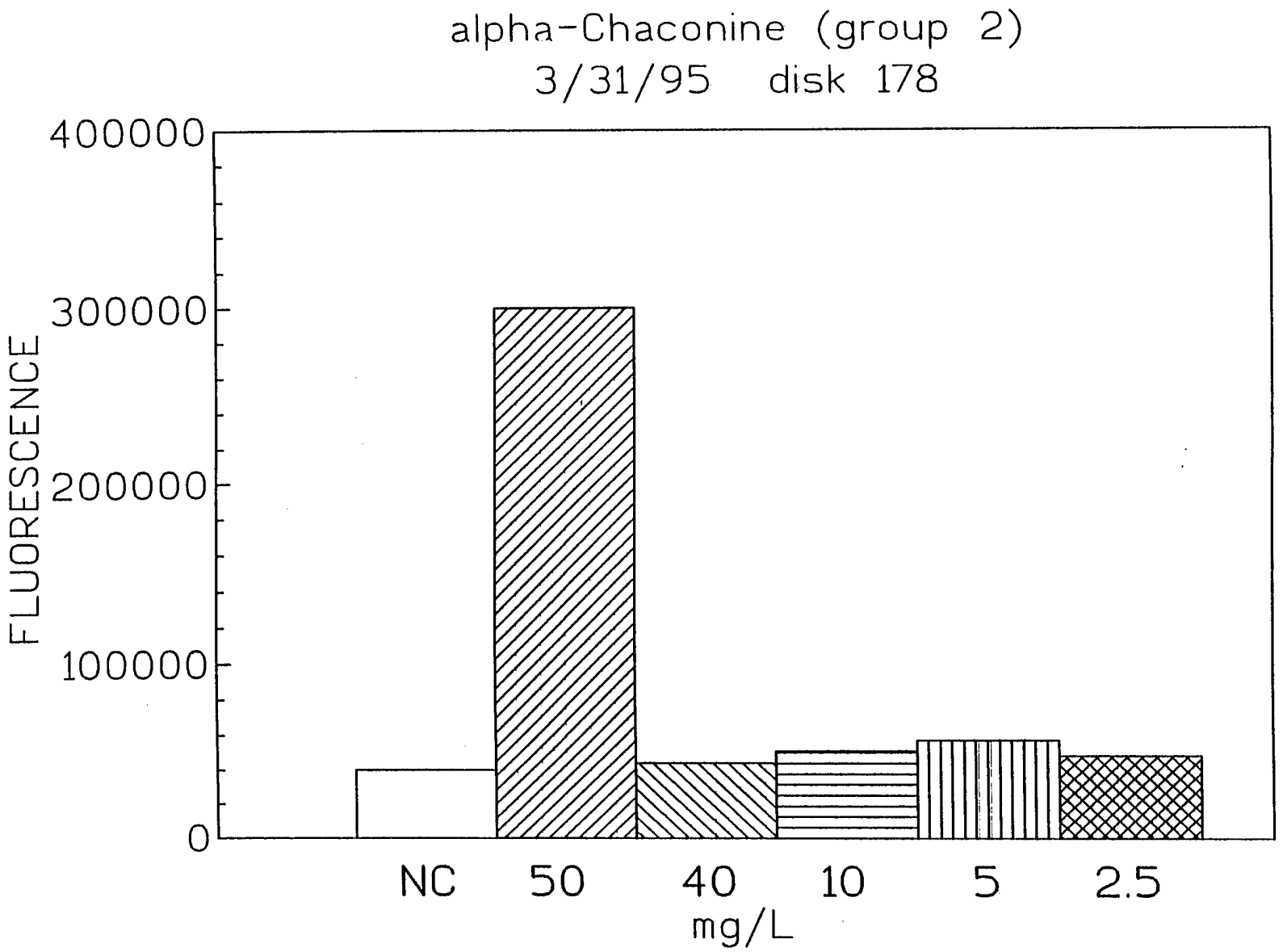

alpha-Chaconine (group 2)
3/31/95 disk 178
400000
300000
200000
100000
0
FLUORESCENCE
NC
50
40
10
5
2.5
mg/L

Figure 5

(30 mg/L stock naphthalene) CONCENTRATION OF NAPHTHALENE	SURVIVAL DAY 0	DAY 3
Negative control	50	45
Positive control (50mg/L a-Chaconine	50	0
2.5 mg/L Naphthalene	50	42
5.0 mg/L Naphthalene	50	42
10.0 mg/L Naphthalene	50	45
15.0 mg/L Naphthalene	50	42
30.0 mg/L Naphthalene	50	48

May 12, 1995

(50 mg/L stock kainic acid) CONCENTRATION OF KAINIC ACID	SURVIVAL DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
Negative control	60	58	56	55	55
Positive control (5mg/L a-Chaconin	40	28	0	0	0
0.2 ml kainic acid	60	58	58	58	58
1.0 ml kainic acid	60	58	58	58	58
2.0 ml kainic acid	60	60	58	58	58
5.0 ml kainic acid	60	56	55	53	53
10 ml kainic acid	60	59	59	59	58

May 31, 1995

50 mg/L kainic acid stock)

CONCENTRATION OF KAINIC ACID	SURVIVAL DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
negative control	50	50	49	47	47
50 mg/L kainic acid	50	50	50	50	49
25 mg/L kainic acid	50	49	48	48	48
12.5 mg/L kainic acid	50	47	47	47	47

May 31, 1995

(50 mg/L kainic acid stock)

CONCENTRATION OF KAINIC ACID	SURVIVAL DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
negative control	50	49	49	49	48
5 mg/L kainic acid	50	50	50	50	49
2.5 mg/L kainic acid	50	50	49	49	49
1 mg/L kainic acid	50	49	48	48	47
0.5 mg/L kainic acid	50	50	48	48	47
0.25 mg/L kainic acid	50	50	49	47	47
0.125 mg/L kainic acid	50	49	47	47	46
0.05 mg/L kainic acid	50	49	47	47	47

June 1, 1995

(50 mg/L colchicine stock)

CONCENTRATION OF COLCHICINE	SURVIVAL DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
ative control	50	49	48	48	48
mg/L colchicine	50	50	50	49	49
2.5 mg/L colchicine	50	50	50	49	49
1 mg/L colchicine	50	50	49	49	49
0.5 mg/L colchicine	50	50	49	49	49
0.25 mg/L colchicine	50	50	50	49	49
0.125 mg/L colchicine	50	50	50	50	50
0.05 mg/L colchicine	50	50	49	49	48

June 1, 1995

(50 mg/L colchicine stock)

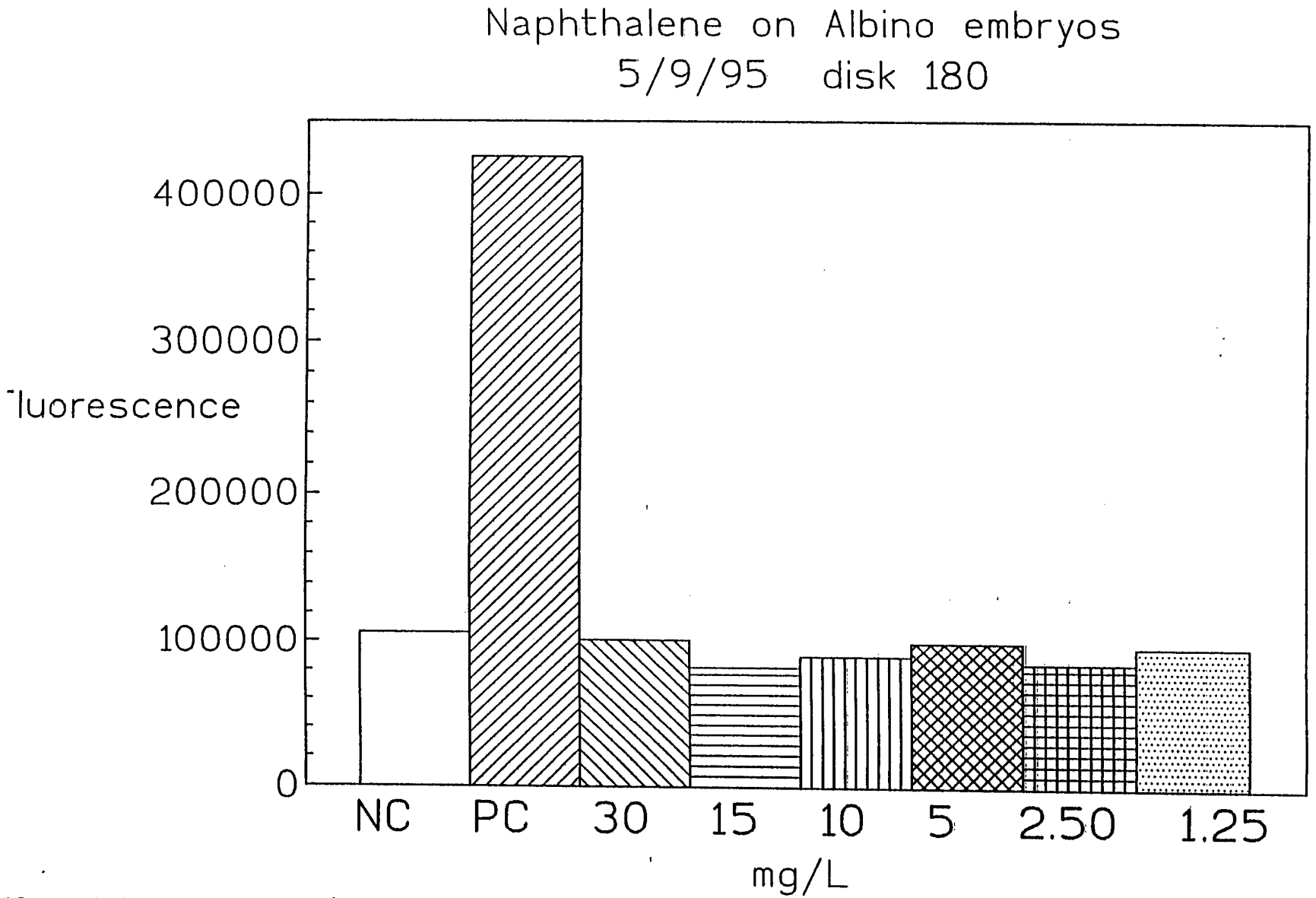
CONCENTRATION OF COLCHICINE	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
ative control	100	99	98	98	9
sitive control (5 mg/L alpha-Chacon	50	42	15	0	
0.2 ml colchicine	100	100	98	96	9
1 ml colchicine	100	99	94	94	9
2 ml colchicine	100	100	99	98	9
5 ml colchicine	100	100	97	96	9
10 ml colchicine	100	100	99	99	9

June 1, 1995

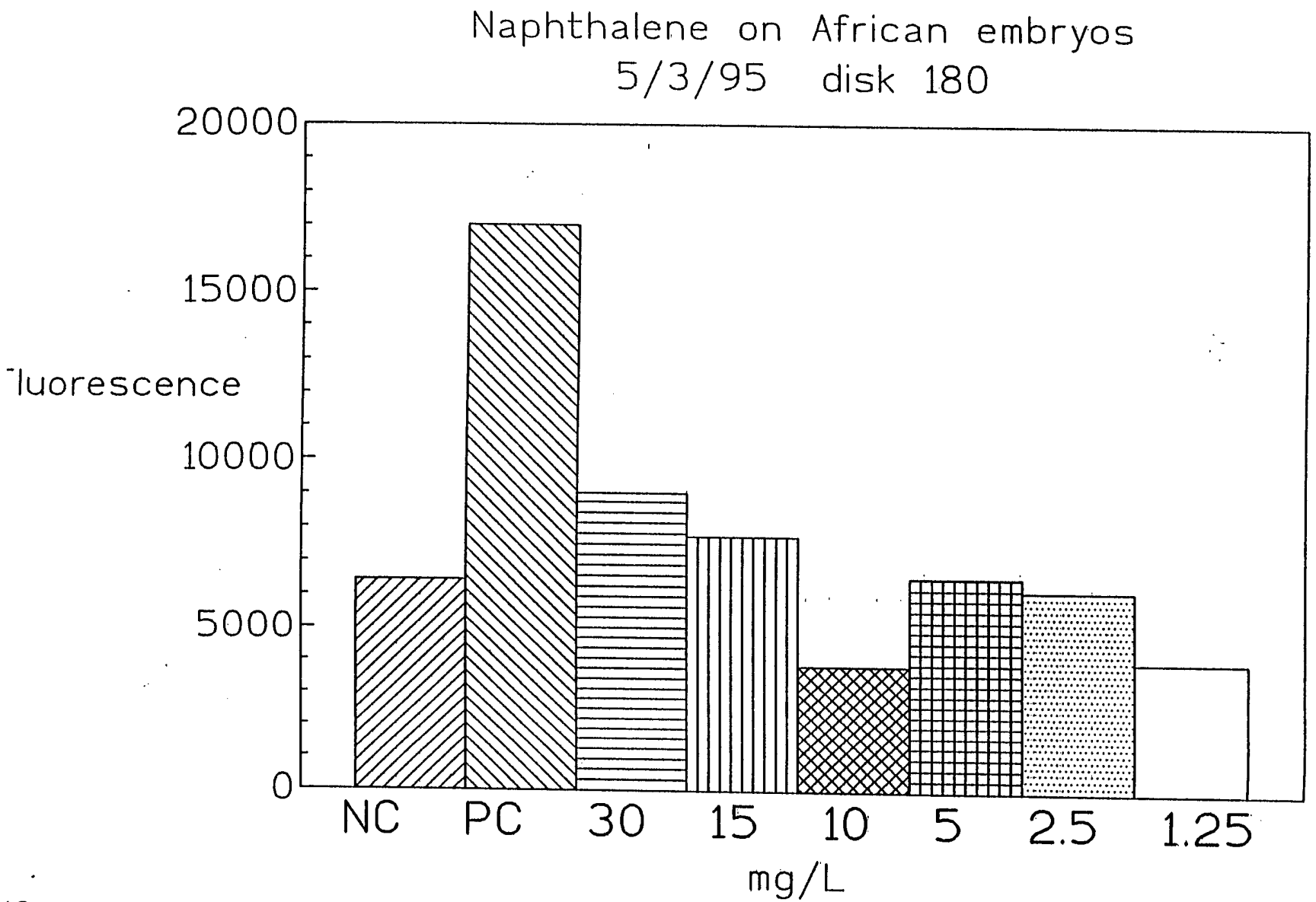
(50 mg/L colchicine stock)

CONCENTRATION OF COLCHICINE	SURVIVAL DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
ative control	25	25	19	18	18
sitive control (50 mg/L a-Chacon	25	25	22	21	21
5 mg/L	25	25	18	17	17
15 mg/L	25	25	21	19	19
25 mg/L	25	25	22	21	19
50 mg/L	25	0	0	0	0

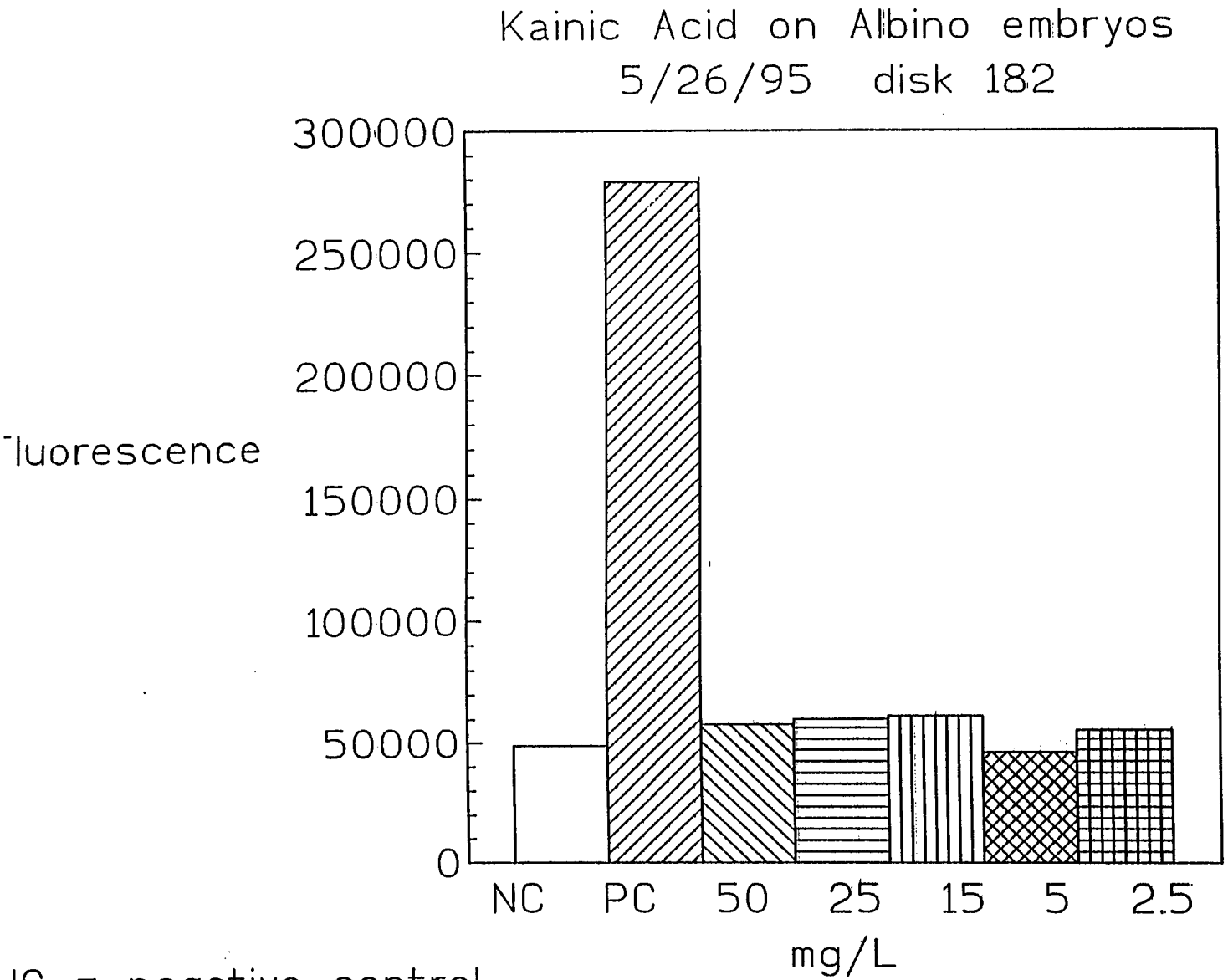
June 8, 1995



IC = fetax solution (negative control)
'C = 50 mg/L a-Chaconine (positive control)



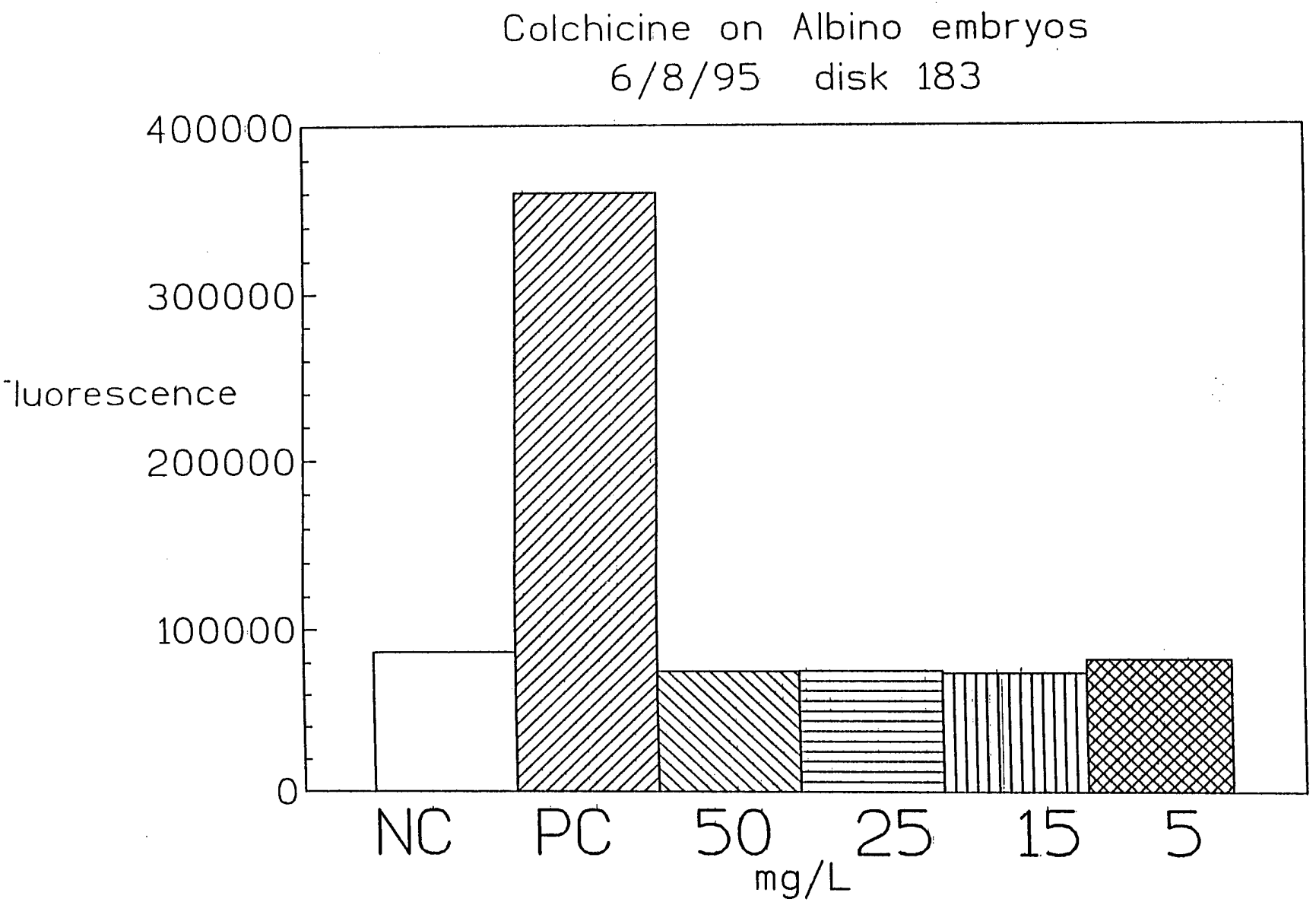
NC = negative control (fetax solution)
PC = postive control (50 mg/L a-Chaconine)



NC = negative control

PC = postive control (50mg/L a-Chaconine)

Figure 8



NC = negative control

PC = postive control (50 mg/L a- Chaconine)

Vinblastine on Normal Embryos

June 29, 1995		Number Survived		
Dish #	CONCENTRATION	DAY 0	DAY4	COMMENTS
15,16	Negative Control	50	46	
13,14	0.1562 mg/L	50	47	
11,12	0.3125 mg/L	50	48	
9,10	0.625 mg/L	50	46	
7,8	1.25 mg/L	50	44	
5,6	2.5 mg/L	50	45	
3,4	5.0 mg/L	50	39	all malformed
1,2	10.0 mg/L	50	37	all malformed

Table 8

Vinblastine on Embryos

7/3/95

DISH #	CONCENTRATION	TOXICANT	DAY 0 (embryos per dish	DAY 4 (96HR.) -SURVIVAL (embryos per dish)
1	10 mg/L	Vinblastine	25	16
2	10 mg/L	Vinblastine	25	21
3	5 mg/L	Vinblastine	25	16
4	5 mg/L	Vinblastine	25	23
5	2.5 mg/L	Vinblastine	25	21
6	2.5 mg/L	Vinblastine	25	23
7	1.25 mg/L	Vinblastine	25	22
8	1.25 mg/L	Vinblastine	25	22
9	0.625 mg/L	Vinblastine	25	24
10	0.625 mg/L	Vinblastine	25	22
11	0.3125 mg/L	Vinblastine	25	23
12	0.3125 mg/L	Vinblastine	25	25
13	0.1562 mg/L	Vinblastine	25	23
14	0.1562 mg/L	Vinblastine	25	24
15	Negative control	FETAX solution	25	22
16	Negative control	FETAX solution	25	24

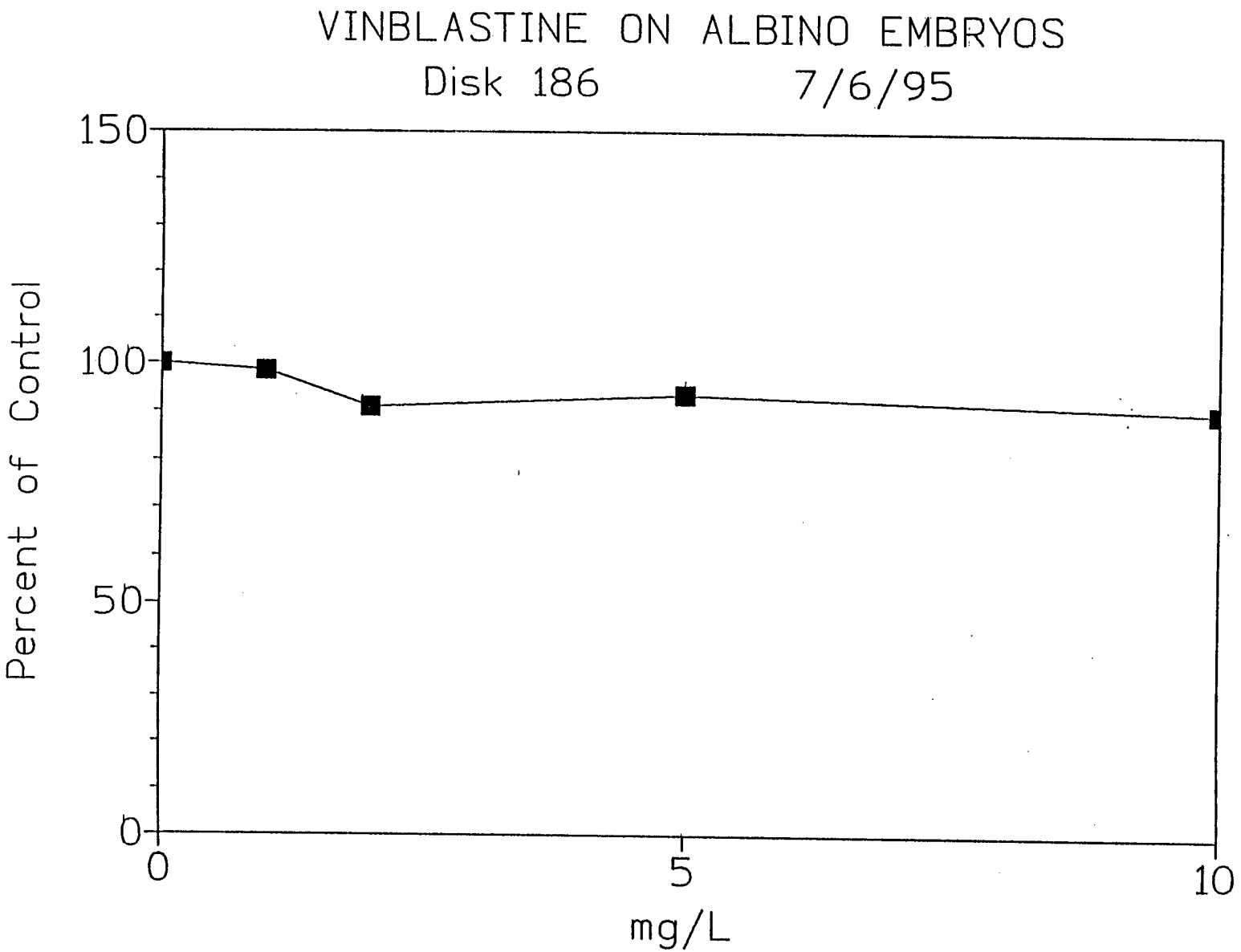

VINBLASTINE ON ALBINO EMBRYOS
Disk 186 7/6/95
150
100
50
0
Percent of Control
0
5
10
mg/L

Figure 10

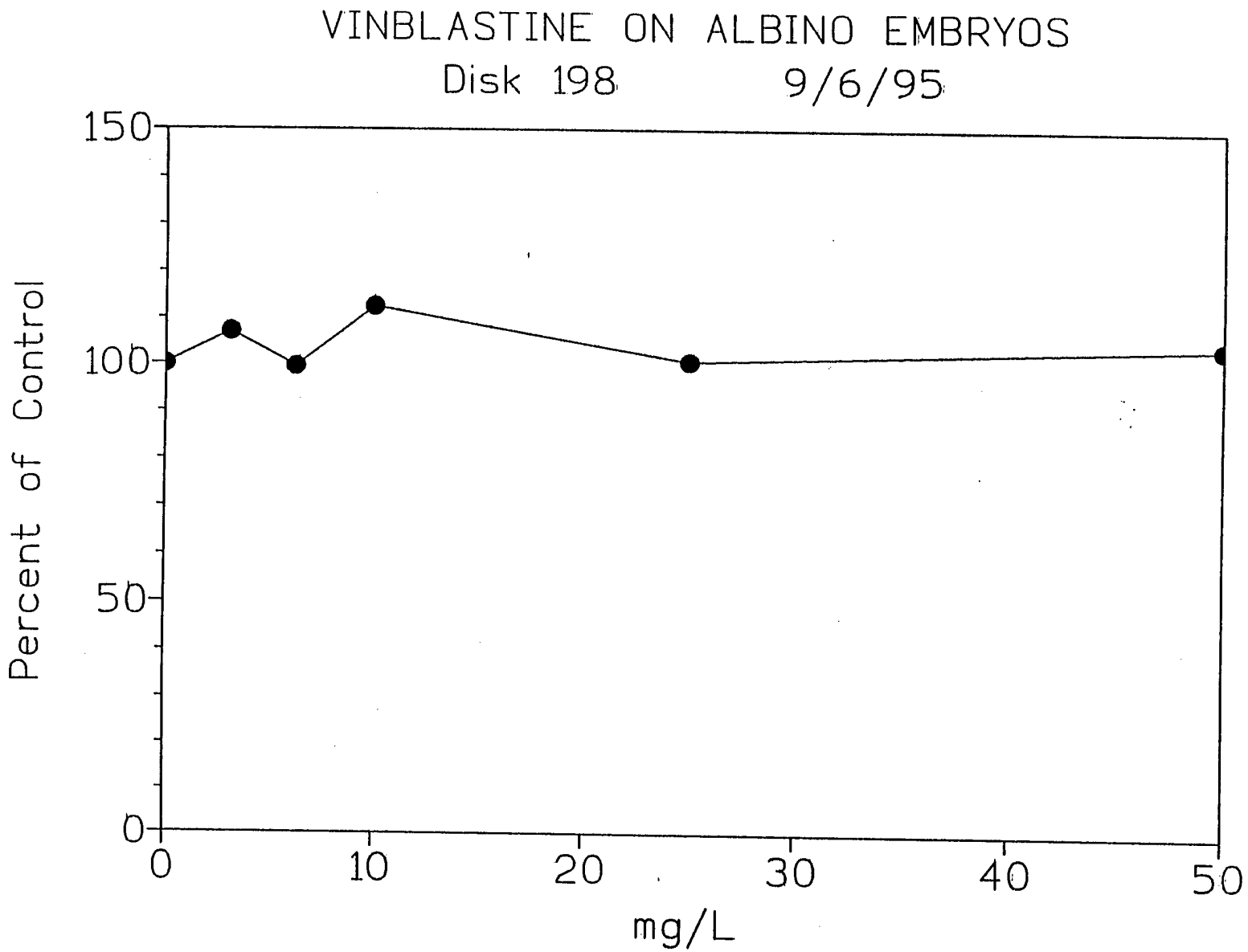
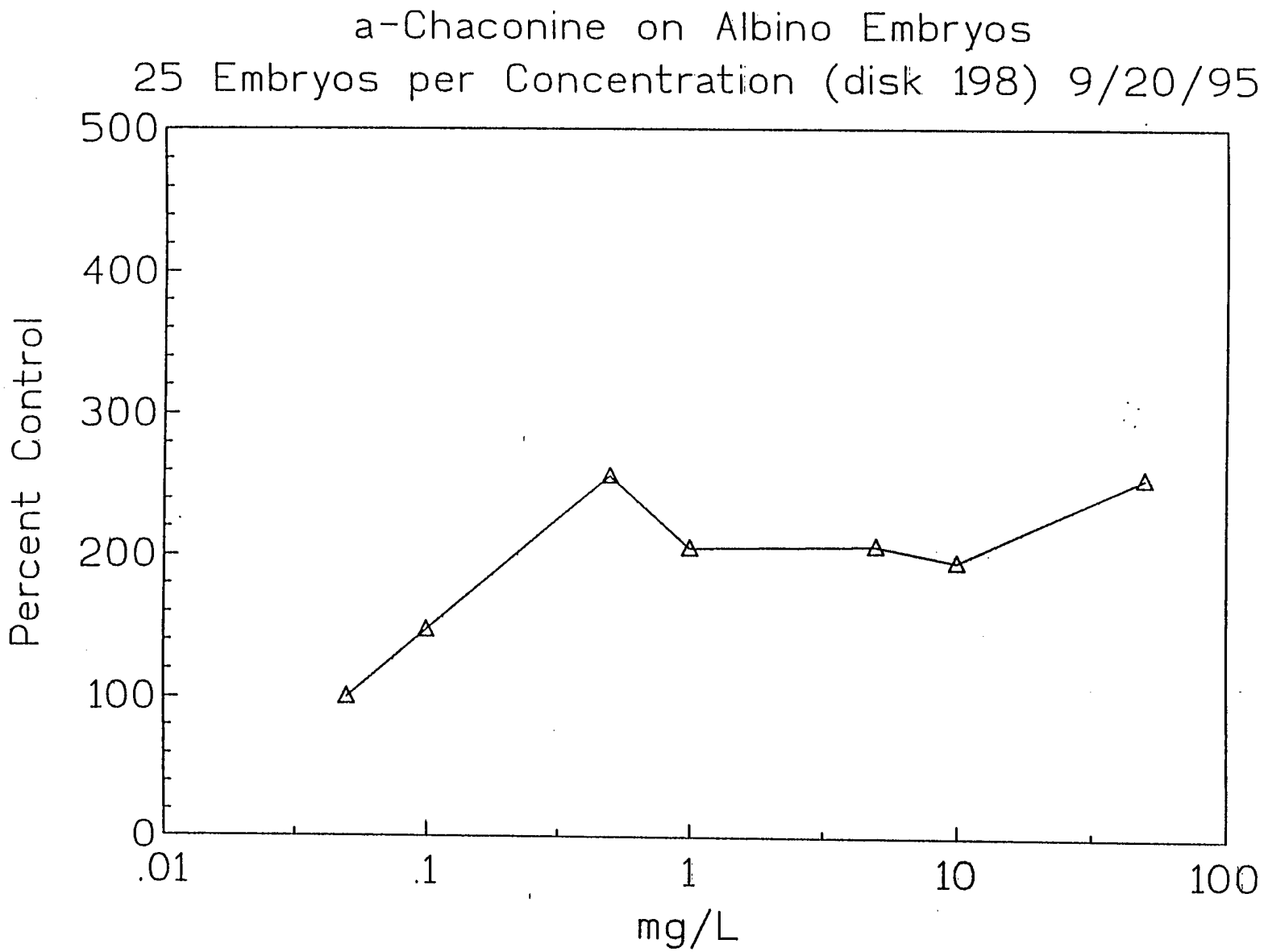

VINBLASTINE ON ALBINO EMBRYOS
Disk 198 9/6/95
Percent of Control
150
100
50
0
0
10
20
30
40
50
mg/L

Figure 11



Transformed (logx, antilog)

Figure 12

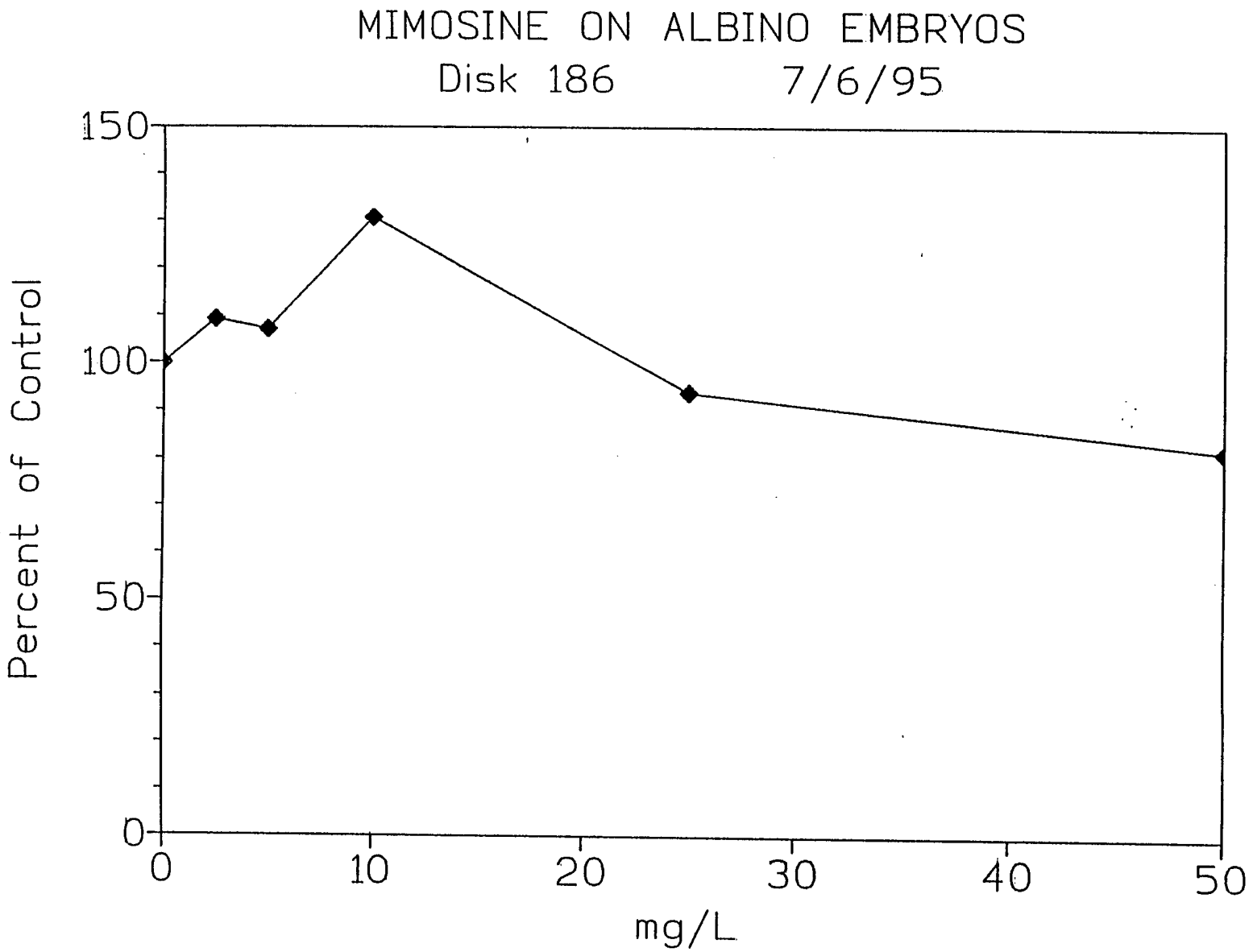

MIMOSINE ON ALBINO EMBRYOS
Disk 186 7/6/95
Percent of Control
150
100
50
0
0
10
20
30
40
50
mg/L

Figure 13

Mimosine on Normal Embryos

June 7, 1995

Dish #	CONCENTRATION	TOXICANT	Number Survived DAY 1	DAY 2	DAY3	DAY 4
1	Negative Control	FETAX solution	25	7	5	4
2	50 mg/L	Mimosine	25	11	9	8
3	25 mg/L	Mimosine	25	5	4	3
4	10 mg/L	Mimosine	25	9	8	7
5	5 mg/L	Mimosine	25	13	12	11
6	2.5 mg/l	Mimosine	25	21	17	16
7	Negative Control	FETAX solution	25	10	9	9
8	50 mg/L	Mimosine	25	7	7	7
9	25 mg/L	Mimosine	25	15	13	13
10	10 mg/L	Mimosine	25	9	6	6
11	5 mg/L	Mimosine	25	11	9	9
12	2.5 mg/L	Mimosine	25	8	8	8

Albizia on Normal Embryos

Albizia on Embryos

June 22, 1995

DISH #	Toxicant	Number Survived Day 0	Day 1	Day 2	Day 3	Day 4
1	Negative Contro	25	25	25	25	23
2	Negative Contro	25	25	25	23	22
3	Negative Contro	25	25	24	23	23
4	Albizia	25	25	25	25	24
5	Albizia	25	25	24	24	22
6	Albizia	25	25	24	24	24

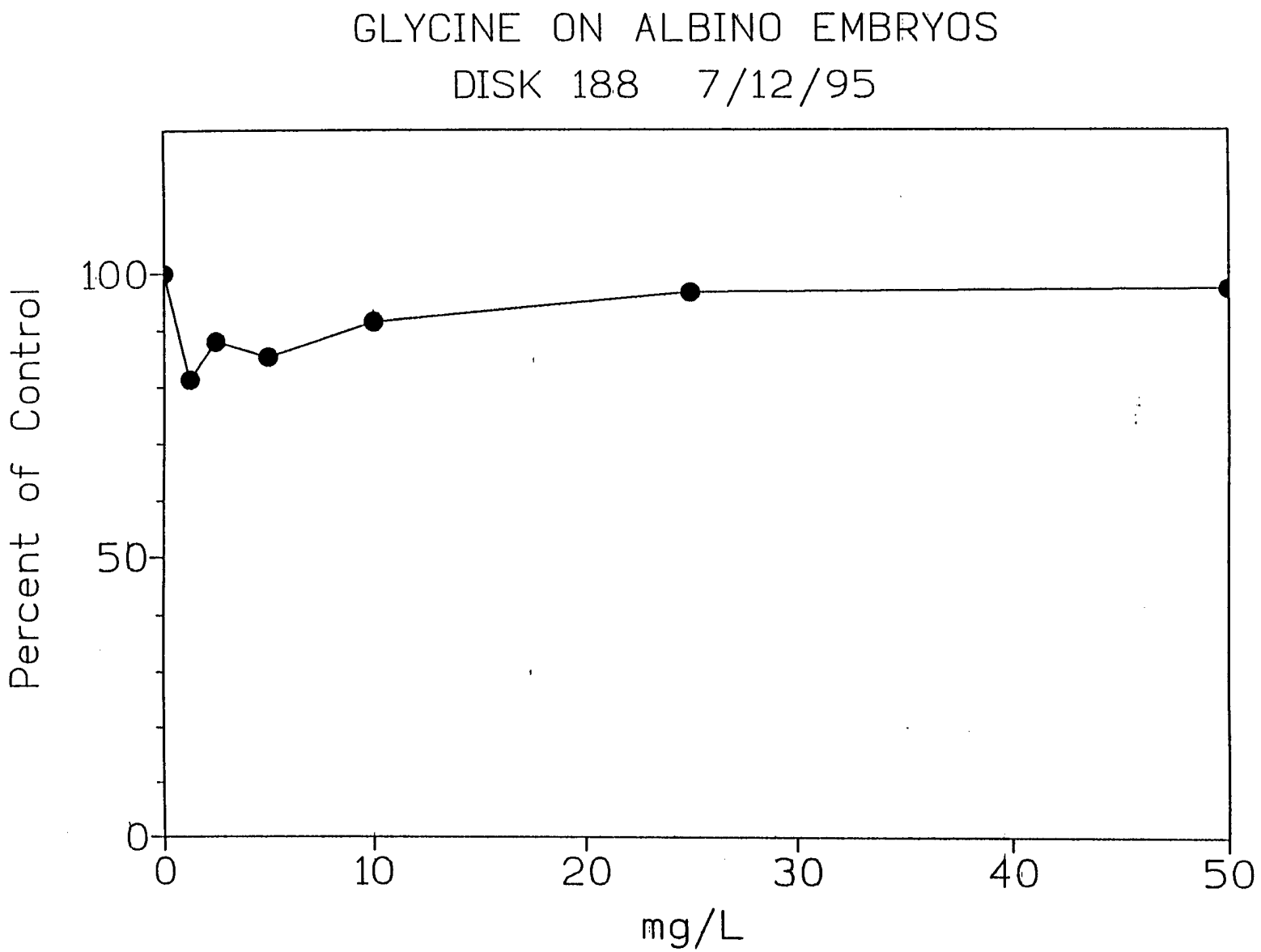

GLYCINE ON ALBINO EMBRYOS
DISK 188 7/12/95
Percent of Control
100
50
0
0
10
20
30
40
50
mg/L

Figure 14

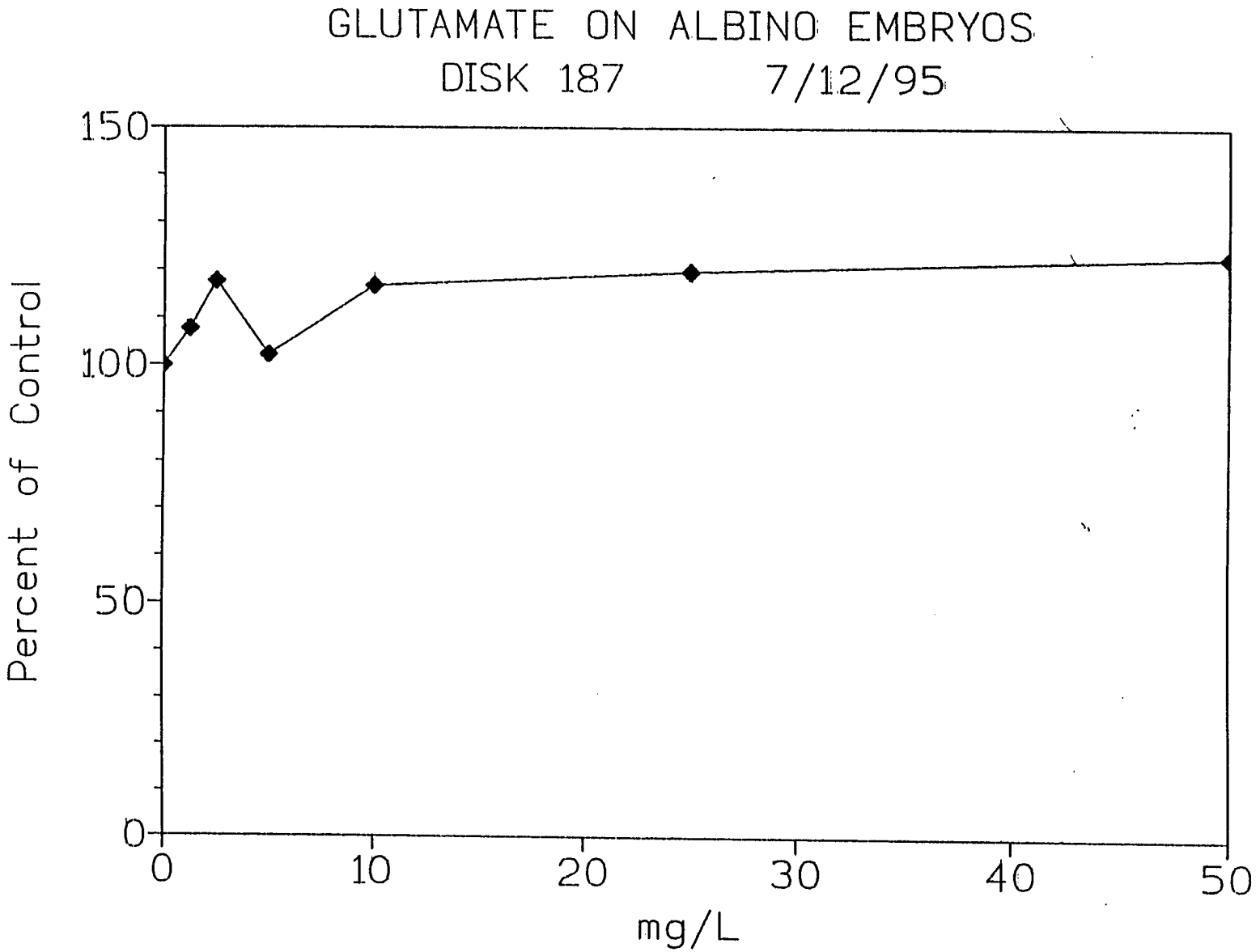

GLUTAMATE ON ALBINO EMBRYOS
DISK 187 7/12/95
Percent of Control
150
100
50
0
0
10
20
30
40
50
mg/L

Figure 15

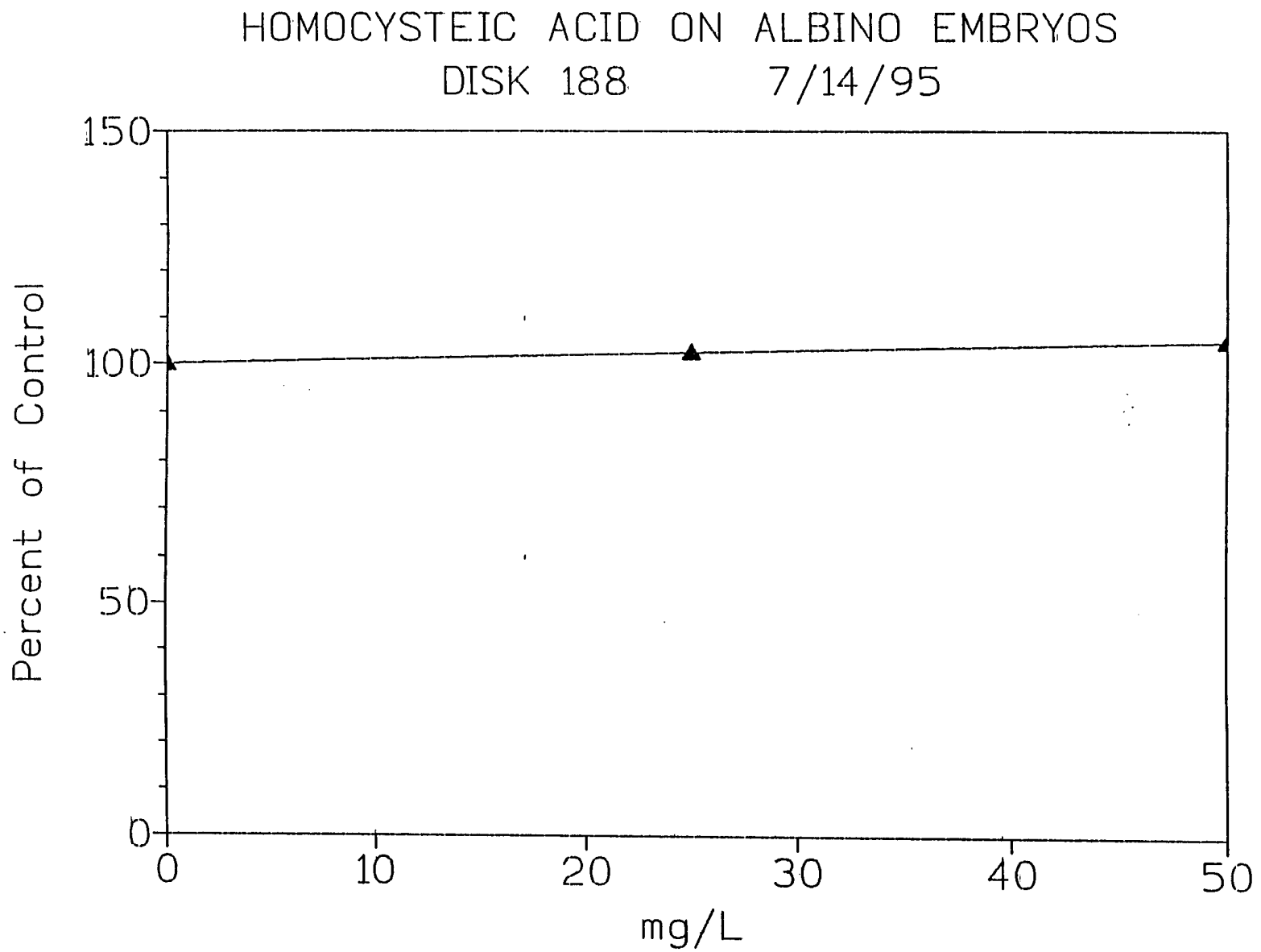

HOMOCYSTEIC ACID ON ALBINO EMBRYOS
DISK 188 7/14/95
Percent of Control
150
100
50
0
0
10
20
30
40
50
mg/L

Figure 16

ALBINO EMBRYOS VS ALPHA-CHACONINE AT LOW CONCENTRATIONS

October 31, 1995

All embryos at stages 1-8 (0+ hrs old).
Dishes 1-6 set up for testing on October 4, 1995.

Dish 1: Negative control for dishes 1-6. 5 albino embryos incubated in 10mL of fetax
Dish 2: 5 albino embryos incubated in 0.5mg/L a-chaconine
Dish 3: 5 albino embryos incubated in 1.0mg/L a-chaconine
Dish 4: 5 albino embryos incubated in 2.5mg/L a-chaconine
Dish 5: 5 albino embryos incubated in 5.0mg/L a-chaconine
Dish 6: 5 albino embryos incubated in 10.0mg/L a-chaconine

October 4, 1995

All embryos at stage 13 (24+ hrs old).

Dish 1: Negative control for dishes 1-6. 5 albino embryos 10mL of fetax for 26hrs and 20uL di-8-Anepps
Dish 2: 5 albino embryos incubated in 0.5mg/L a-chaconine for 26hrs and 20uL di-8-Anepps for 30min
Dish 3: 5 albino embryos incubated in 1.0mg/L a-chaconine for 26hrs and 20uL di-8-Anepps for 30 min
Dish 4: 5 albino embryos incubated in 2.5mg/L a-chaconine for 26hrs and 20uL di-8-Anepps for 30 min
Dish 5: 5 albino embryos incubated in 5.0mg/L a-chaconine for 26hrs and 20uL di-8-Anepps for 30min
Dish 6: 5 albino embryos incubated in 10.0mg/L a-chaconine for 26hrs and 20uL di-8-anepps for 30min

Photos taken of dishes 1-6 with 1000 ASA film, 5X photo eyepiece, 10X objective a B-filter, and a Nikon 35mm camera attached to a Nikon camerahead,

Dishes 7-13 set up for testins on October 5. 1994.

Dish 7: Negative control for dishes 7-13. 5 albino embryos incubated in 10mL of fetax
Dish 8: 5 albino embryos incubated in fetax for 26hrs then 0.25mg/L a-chaconine
Dish 9: 5 albino embryos incubated in fetax for 26hrs then 0.5mg/L a-chaconine
Dish 10: 5 albino embryos incubated in fetax for 26hrs then 1.0mg/L a-chaconine
Dish 11: 5 albino embryos incubated in fetax for 26hrs then 2.5mg/L a-chaconine
Dish 12: 5 albino embryos incubated in fetax for 26hrs then 5.0mg/L a-chaconine
Dish 13: 5 albino embryos incubated in fetax for 26hrs then 10.0mg/L a-chaconine

October 5, 1995

All embryos are 48+ hrs old.

Dish 1: Negative control for dishes 1-6. 5 albino embryos incubated in 10mL of fetax for 51hrs and 20uL di-8-Anepps for 25hrs
Dish 2: 5 albino embryos incubated in 0.5 mg/L a-chaconine for 51hrs and 20uL di-8-Anepps for 25hrs
Dish 3: 5 albino embryos Incubated in 1.0 mg/L a-chaconine for 51hrs and 20uL di-8-Anepps for 25hrs
Dish 4: 5 albino embryos incubated in 2.5mg/L a-chaconine for 51hrs and 20uL di-8-Anepps for 25hrs
Dish 5: 5 albino embryos incubated in 5.0mg/L a-chaconine for 51hrs and 20uL di-8-Anepps for 25hrs
Dish 6: 5 albino embryos incubated in 10.0mg/L a-chaconine for 51hrs and 20uL di-8-Anepps for 25hrs

Dish 7: Negative control for dishes 7-13. 5 albino embryos incubated in 10mL of fetax for 51hrs and 20uL di-8-Anepps

Dish 8: 5 albino embryos incubated in fetax for 26hrs then 0.25 mg/L a-chaconine for 25hrs and 20uL di-8-Anepps for 30min
Dish 9: 5 albino embryos incubated in fetax for 26hrs then 0.5 mg/L a-chaconine for 25hrs and 20uL di-8-Anepps for 30 min
Dish 10: 5 albino embryos incubated in fetax for 26hrs then 1.0 mg/L a-chaconine for 25hrs and 20uL di-8-Anepps for 30 min
Dish 11: 5 albino embryos incubated in fetax for 26hrs then 2.5 mg/L a-chaconine for 25hrs and 20uL di-8-Anepps for 30 min
Dish 12: 5 albino embryos incubated in fetax for 26hrs then 5.0 mg/L a-chaconine for 25hrs and 20uL di-8-Anepps for 30 min
Dish 13: 5 albino embryos incubated in fetax for 26hrs then 10.0 mg/L a-chaconine for 25hrs and 20uL di-8-Anepps for 30 min

Photos taken of dishes 1-6 with 1000 ASA film, 5X photo eyepiece, 10X objective, a B-filter and a Nikon 35mm camera attached to a Nikon camerahead.

<u>Dishes were set up for testing on October 6, 1995.</u>

Dish 14: 5 albino embryos incubated in fetax for 52hrs and 0.25 mg/L a-chaconine
Dish 15: 5 albino embryos incubated in fetax for 52hrs and 0.5 mg/L a-chaconine
Dish 16: 5 albino embryos incubated in fetax for 52hrs and 1.0 mg/L a-chaconine
Dish 17: 5 albino embryos incubated in fetax for 52hrs and 2.5 mg/L a-chaconine

Dish 18: 5 albino embryos incubated in fetax for 52hrs and 5.0 mg/L a-chaconine
Dish 19: 5 albino embryos incubated in fetax for 52hrs and 10.0 mg/L a-chaconine

October 6, 1995

All embryos are 72+ hrs old.

Dish 7: Negative control for dishes 7-13. 5 albino embryos incubated in 10mL of fetax for 72hrs and redyed with 20uL di-8-Anepps for 30min
Dish 10: 5 albino embryos incubated in fetax for 26hrs then 1.0mg/L a-chaconine for 46 hrs and redyed with 20uL di-8-Anepps for 30min
Dish 11: 5 albino embryos incubated in fetax for 26hrs then 2.5mg/L a-chaconine for 46hrs and redyed with 20uL di-8 Anepps for 30min

Dish 14: 5 albino embryos incubated in fetax for 52hrs then 0.25mg/L a-chaconine for 24hrs and 20uL di-8-Anepps for 30min
Dish 15: 5 albino embryos incubated in fetax for 52hrs and 0.5 mg/L a-chaconine for 24hrs and 20uL di-8-Anepps for 30 min
Dish 16: 5 albino embryos incubated in fetax for 52hrs and 1.0mg/L a-chaconine for 24hrs and 20uL di-8-Anepps for 30 min
Dish 17: 5 albino embryos incubated in fetax for 52hrs and 2.5mg/L a-chaconine for 24hrs and 20uL di-8-Anepps for 30min
Dish 18: 5 albino embryos incubated in fetax for 52hrs and 5.0mg/L a-chaconine for 24hrs and 20uL di-8-Anepps for 30 min
Dish 19: 5 albino embryos incubated in fetax for 52hrs and 10.0mg/l a-chaconine for 24hrs and 20uL di-8-Anepps for 30 min

Photos taken of dishes 1-6 with 1000 ASA film, 5X photo eyepiece, 10X objective, a B-filter, and a Nikon 35mm camera attached to a Nikon camerahead.

ACRYLAMIDE on Embryos

October 31, 1995			Number Survived	
DISH#	CONCENTRATION	DAY 0	DAY 1	DAY 2
1	Negative control	20	20	20
2	Negative control	20	20	19
3	10 mg/L acrylamide	20	19	19
4	10 mg/L acrylamide	20	20	19
5	5 mg/L acrylamide	20	20	19
6	5 mg/L acrylamide	20	19	19
7	2.5 mg/L acrylamide	20	20	20
8	2.5 mg/L acrylamide	20	17	17
9	1 mg/L acrylamide	20	19	19
10	1 mg/L acrylamide	20	20	19

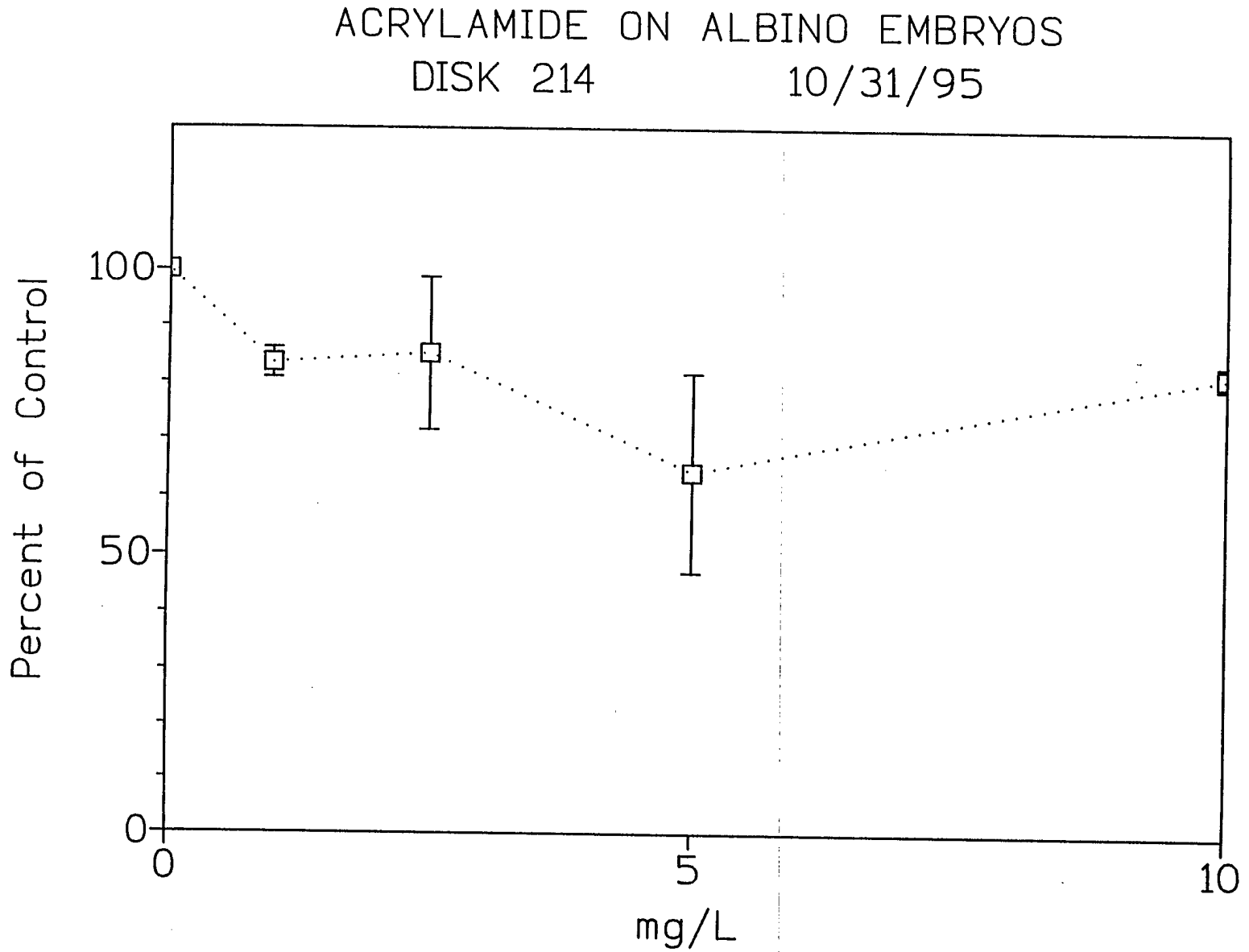

ACRYLAMIDE ON ALBINO EMBRYOS
DISK 214
10/31/95
Percent of Control
100
50
0
0
5
10
mg/L

Figure 17

alpha-Chaconine Survival Experiment

1st Day Embryos

CONCENTRATION	Nov. 7, 1995	Nov. 8, 1995	Nov. 9, 1995	Nov. 10,1995
Negative Control	6	6	6	6
0.1 mg/L a-Chac.	6	6	6	6
1 mg/L a-Chac.	6	5	5	5
2 mg/L a-Chac.	6	6	6	6
5 mg/L a-Chac.	6	4	0	0

set up Nov. 7, 1995

2nd Day Embryos

CONCENTRATION	Nov. 8, 1995	Nov. 9, 1995	Nov. 10, 1995
Negative Control	6	6	6
0.1 mg/L a-Chac.	6	6	6
1 mg/L a-Chac	6	6	6
2 mg/L a-Chac.	6	6	6
5 mg/L a-Chac.	6	0	0

set up Nov. 8, 1995

3rd Day Embryos

CONCENTRATION	Nov. 9, 1995	Nov. 10, 1995
Negative Control	6	6
0.1 mg/L a-Chac.	6	6
1 mg/L a-Chac.	6	6
2 mg/L a-Chac	6	6
5 mg/L a-Chac.	6	0

set up Nov. 9, 1995

TCAOB & TCAB on Embryos (1% EtOH)

ovember 15,1995

DISH#	CONCENTRATION	Number Survived DAY 0	DAY 1	DAY 2	DAY 3	DAY 5
1	Negative control	25	23	22	22	0
2	Negative control	25	23	23	22	0
3	Negative control	25	25	24	21	0
4	200 ug/L TCAOB	25	23	22	21	0
5	200 ug/L TCAOB	25	23	23	22	22
6	200 ug/L TCAOB	25	23	23	22	0
7	100 ug/L TCAOB	25	24	24	21	17
8	100 ug/L TCAOB	25	22	20	19	0
9	100 ug/L TCAOB	25	25	24	23	0
10	50 ug/L TCAOB	25	25	22	21	15
11	50 ug/L TCAOB	25	24	24	22	0
12	50 ug/L TCAOB	25	23	23	21	7
13	25 ug/L TCAOB	25	23	22	22	14
14	25 ug/L TCAOB	25	24	24	20	0
15	25 ug/L TCAOB	25	25	24	23	0
16	Negative control	25	24	24	24	0
17	Negative control	25	25	25	23	0
18	Negative control	25	23	23	23	21
19	200 ug/L TCAB	25	24	23	22	17
20	200 ug/L TCAB	25	23	23	22	19
21	200 ug/L TCAB	25	21	19	19	16
22	100 ug/L TCAB	25	22	22	20	0
23	100 ug/L TCAB	25	25	25	25	0
24	100 ug/L TCAB	25	21	20	20	0
25	50 ug/L TCAB	25	24	23	22	0
26	50 ug/L TCAB	25	25	24	24	22
27	50 ug/L TCAB	25	24	24	24	16
28	25 ug/L TCAB	25	24	23	17	0
29	25 ug/L TCAB	25	24	24	24	0
30	25 ug/L TCAB	25	24	24	24	0

1% EtOH was used to dissolve the TCAB & TCAOB.

...YLAMIDE on Embryos

November 15, 1995			Number Survived	
DISH#	CONCENTRATION	DAY 0	DAY 1	DAY 2
1	Negative control	25	24	24
2	Negative control	25	24	23
3	10 mg/L acrylamide	25	22	21
4	10 mg/L acrylamide	25	25	24
5	5 mg/L acrylamide	25	23	22
6	5 mg/L acrylamide	25	24	24
7	2.5 mg/L acrylamide	25	24	23
8	2.5 mg/L acrylamide	25	22	22
9	1 mg/L acrylamide	25	25	25
10	1 mg/L acrylamide	25	25	25

TRIMETHYLTIN on Embryos

November 15, 1995		Number Survived			
DISH#	CONCENTRATION	DAY 0	DAY 1	DAY 2	DAY 3
1	10 mg/L TMT	25	22	0	0
2	10 mg/L TMT	25	25	19	0
3	10 mg/L TMT	25	25	24	0

short trial run of TMT

Table 18

TRIMETHYLTIN on Embryos

ecember 5, 1995			Number Survived			
,ISH#	CONCENTRATION	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
1	Negative control	15	14	14	14	14
2	Negative control	15	14	14	14	14
3	Negative control	15	15	15	15	15
4	Negative control	15	14	14	13	13
5	1 mg/L TMT	15	15	15	1	1
6	1 mg/L TMT	15	15	14	1	1
7	1 mg/L TMT	15	15	15	1	1
8	1 mg/L TMT	15	15	14	1	1
9	10 mg/L TMT	15	15	0	0	0

* 1mg/L all deformed

TRIMETHYLTIN on Embryos

December 13, 1995		Number Survived					
DISH#	CONCENTRATION	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	Negative control	10	10	10	10	10	1
2	1 mg/L TMT	10	10	10	10	10	
3	0.5 mg/L TMT	10	10	10	9	10	
4	0.2 mg/L TMT	10	10	10	10	10	1
5	0.1 mg/L TMT	10	10	10	10	10	1
6	0.05 mg/L TMT	10	10	10	10	10	1
7	0.01 mg/L TMT	10	10	10	10	10	1

* 1mg/L and 0.5 mg/L all deformed

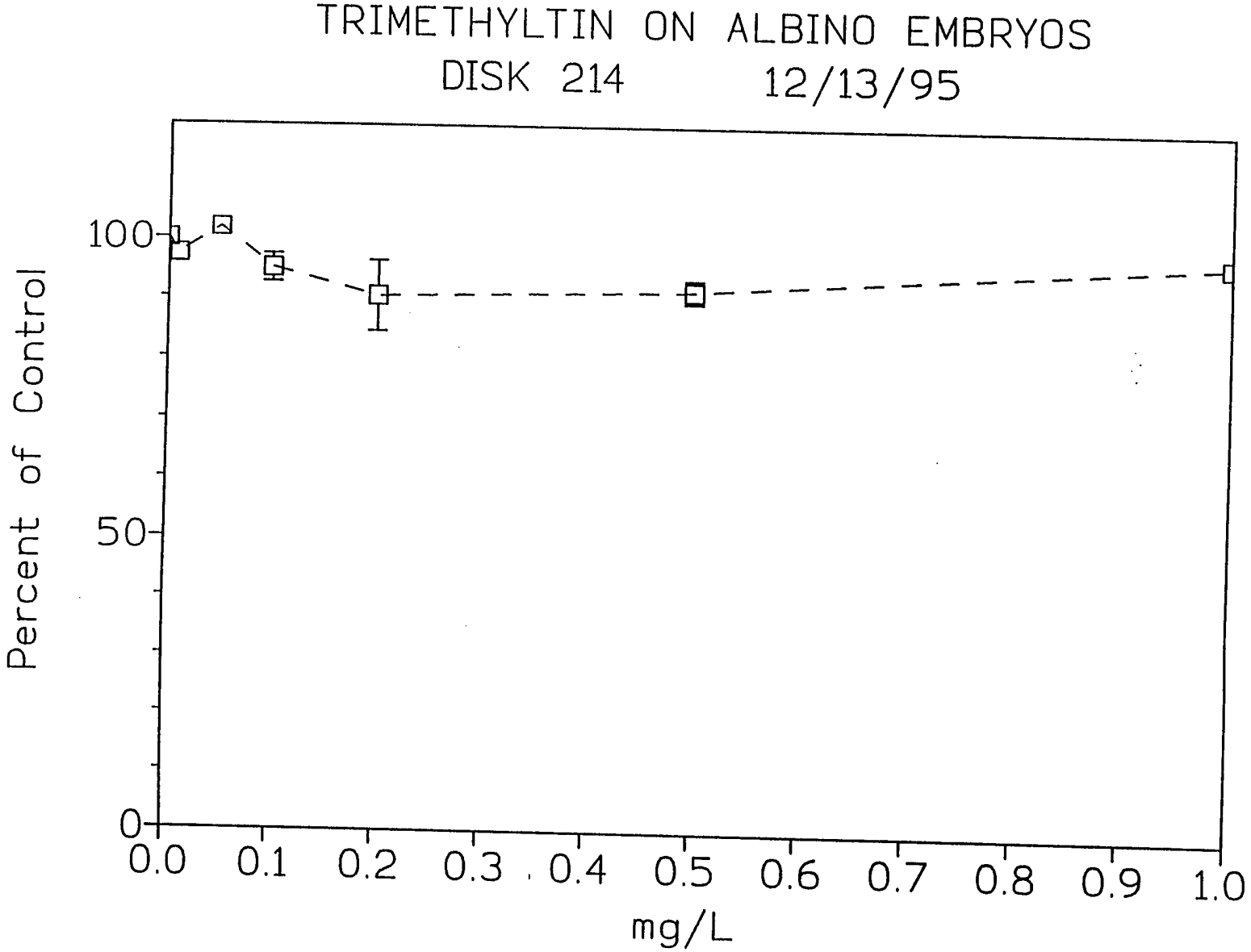

TRIMETHYLTIN ON ALBINO EMBRYOS
DISK 214 12/13/95
Percent of Control
100
50
0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
mg/L

Figure 18

TRIMETHYLTIN on Embryos

December 14, 1995		Number Survived				
DISH#	CONCENTRATION	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
1	Negative control	10	10	10	10	10
2	1 mg/L TMT	10	10	10	10	10*
3	0.5 mg/L TMT	10	10	10	9	8*
4	0.2 mg/L TMT	10	10	10	10	10
5	0.1 mg/L TMT	10	10	10	10	10
6	0.05 mg/L TMT	10	10	10	10	10
7	0.01 mg/L TMT	10	10	10	10	10

* 1mg/L and 0.5 mg/L all deformed embryos are 24 hrs. old

TRIMETHYLTIN on Embryos

December 15, 1995

DISH#	CONCENTRATION	Number Survived DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
1	Negative control	10	10	10	10	9
2	1 mg/L TMT	10	10	10	10	10*
3	0.5 mg/L TMT	10	10	10	10	10*
4	0.2 mg/L TMT	10	10	10	10	10
5	0.1 mg/L TMT	10	10	10	10	10
6	0.05 mg/L TMT	10	10	10	10	10
7	0.01 mg/L TMT	10	10	10	10	10

* 1mg/L and 0.5 mg/L all deformed embryos are 48 hrs. old

TRIMETHYLTIN on Embryos

December 18, 1995

DISH#	CONCENTRATION	Number Survived DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
1	Negative control	25	25	25	25	
2	1 mg/L TMT	25	25	25	25*	
3	0.5 mg/L TMT	25	25	25	25*	
4	0.2 mg/L TMT	25	25	24	24	
5	0.1 mg/L TMT	25	25	25	25	
6	0.05 mg/L TMT	25	25	25	25	
7	0.01 mg/L TMT	25	25	25	25	
8	Negative control	25	25	22	22	
9	1 mg/L TMT	25	25	25	25*	
10	0.5 mg/L TMT	25	24	22	22*	
11	0.2 mg/L TMT	25	25	25	25	
12	0.1 mg/L TMT	25	25	25	25	
13	0.05 mg/L TMT	25	25	25	25	
14	0.01 mg/L TMT	25	25	25	25	

* 1mg/L and 0.5 mg/L all deformed

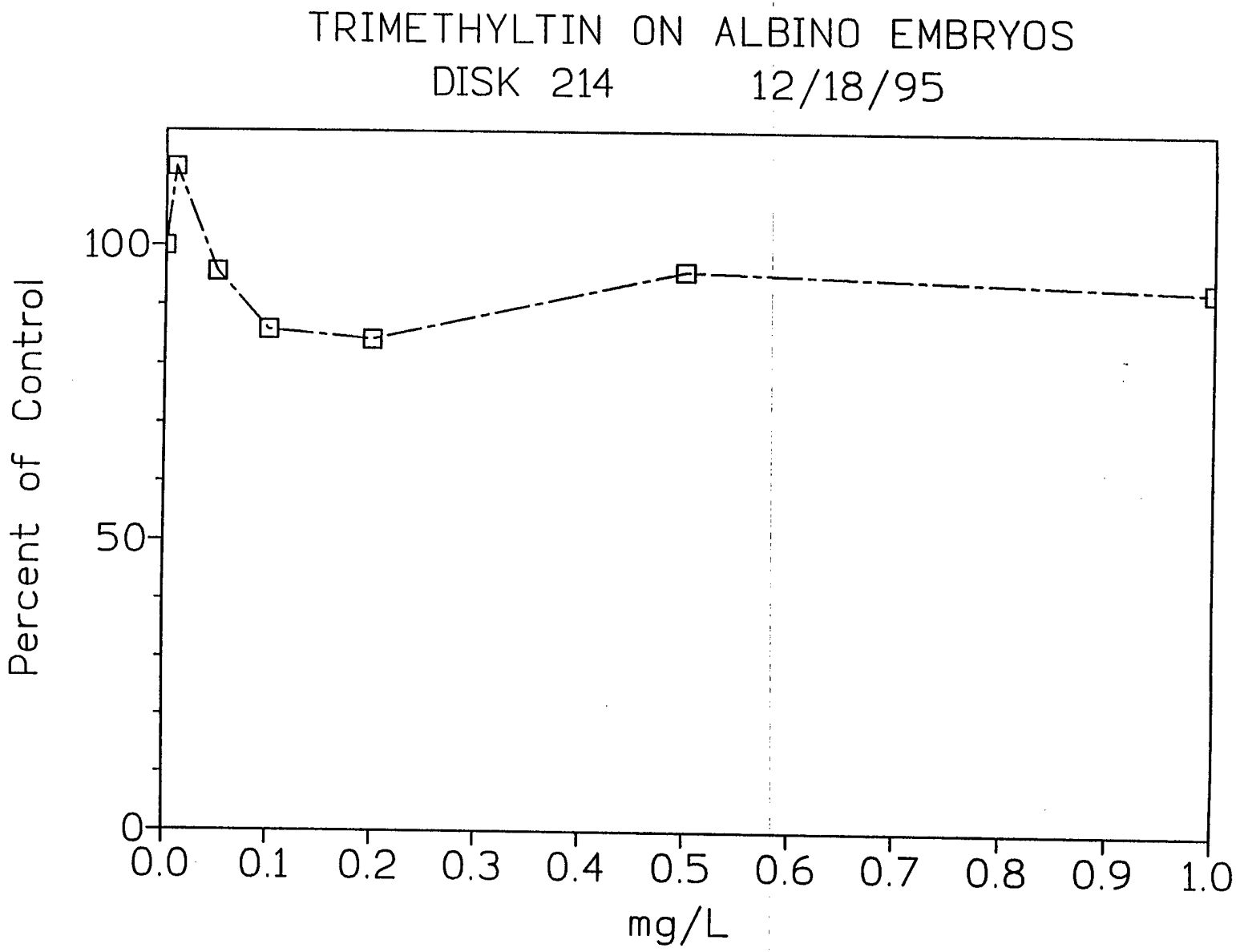

TRIMETHYLTIN ON ALBINO EMBRYOS
DISK 214 12/18/95
Percent of Control
100
50
0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
mg/L

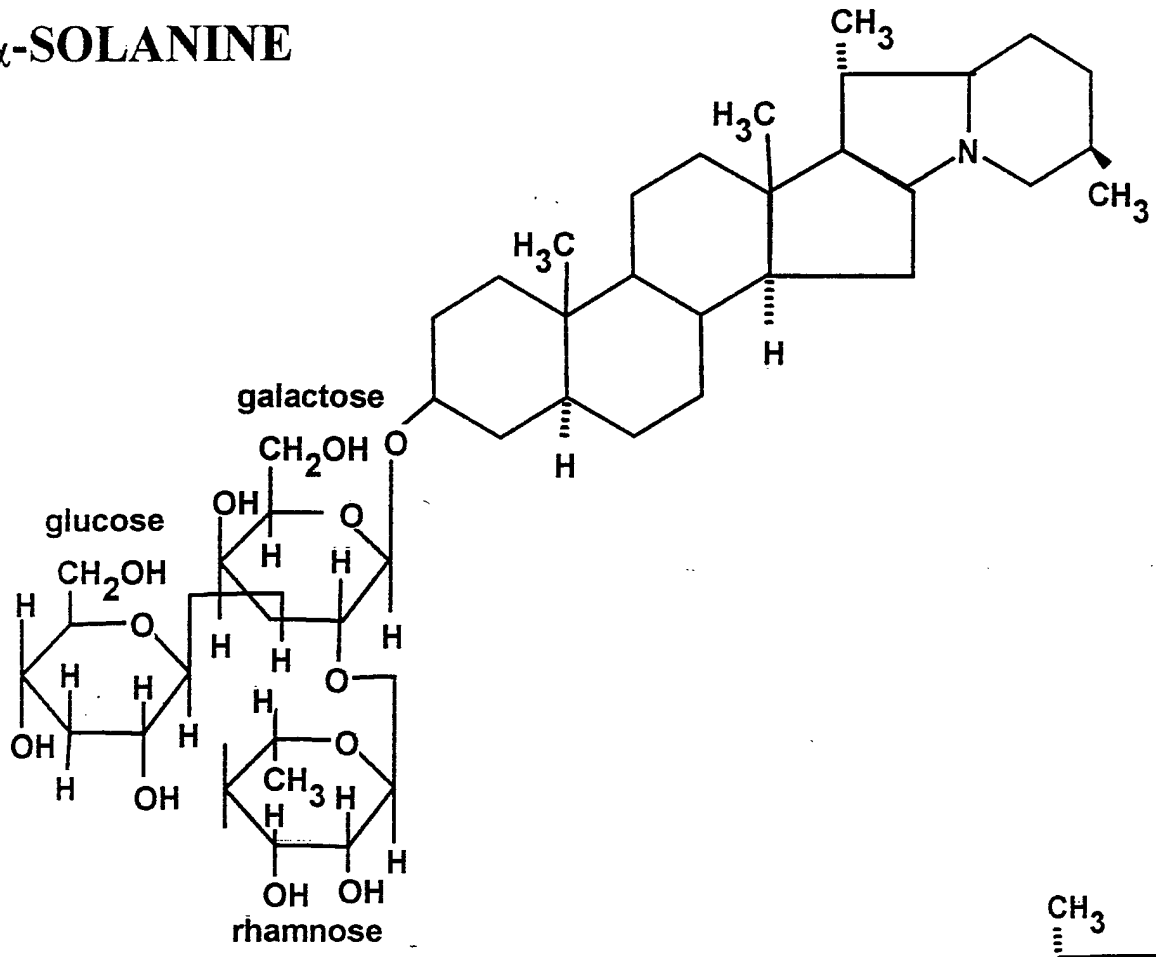
Figure 19

TCAOB & TCAB on Embryos (1% EtOH)

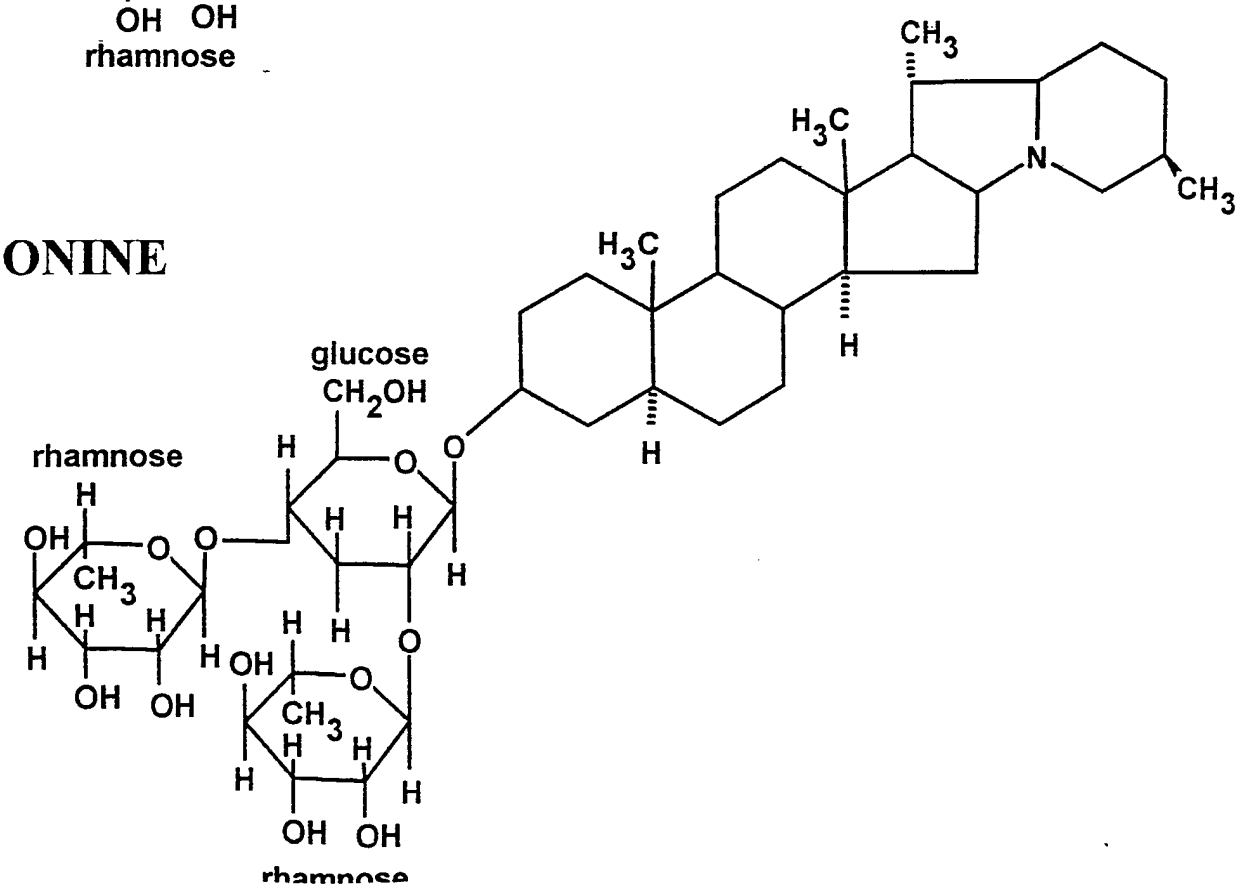
December 21, 1995		Number Survived		
DISH#	CONCENTRATION	DAY 0	DAY 1	DAY 2-4
1	Negative control	25	25	0
2	200 ug/L TCAOB	25	24	0
3	100 ug/L TCAOB	25	25	0
4	50 ug/L TCAOB	25	25	0
5	25 ug/L TCAOB	25	24	0
6	10 ug/L TCAOB	25	23	0
7	Negative control	25	25	0
8	200 ug/L TCAOB	25	24	0
9	100 ug/L TCAOB	25	25	0
10	50 ug/L TCAOB	25	25	0
11	25 ug/L TCAOB	25	24	0
12	10 ug/L TCAOB	25	24	0
13	Negative control	25	25	0
14	200 ug/L TCAB	25	24	0
15	100 ug/L TCAB	25	23	0
16	50 ug/L TCAB	25	25	0
17	25 ug/L TCAB	25	25	0
18	10 ug/L TCAB	25	25	0
19	Negative control	25	25	0
20	200 ug/L TCAB	25	24	0
21	100 ug/L TCAB	25	25	0
22	50 ug/L TCAB	25	24	0
23	25 ug/L TCAB	25	23	0
24	10 ug/L TCAB	25	25	0

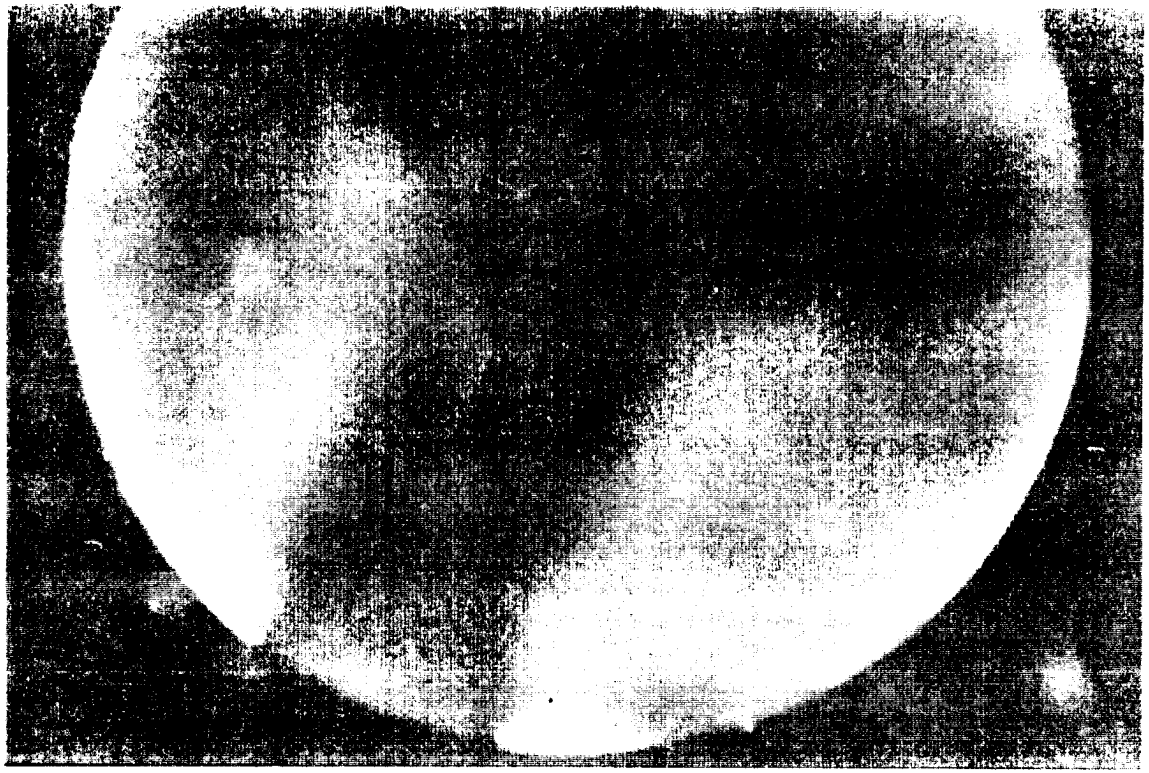
1% EtOH was used to dissolve the TCAB & TCAOB.

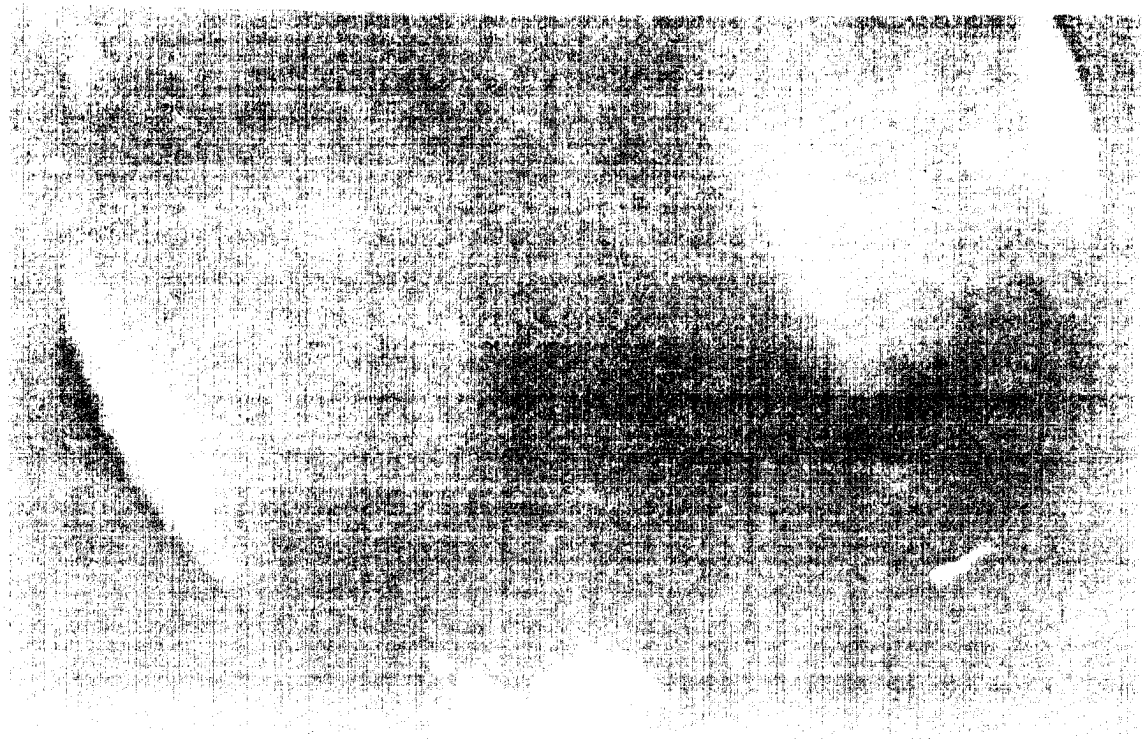
α-SOLANINE

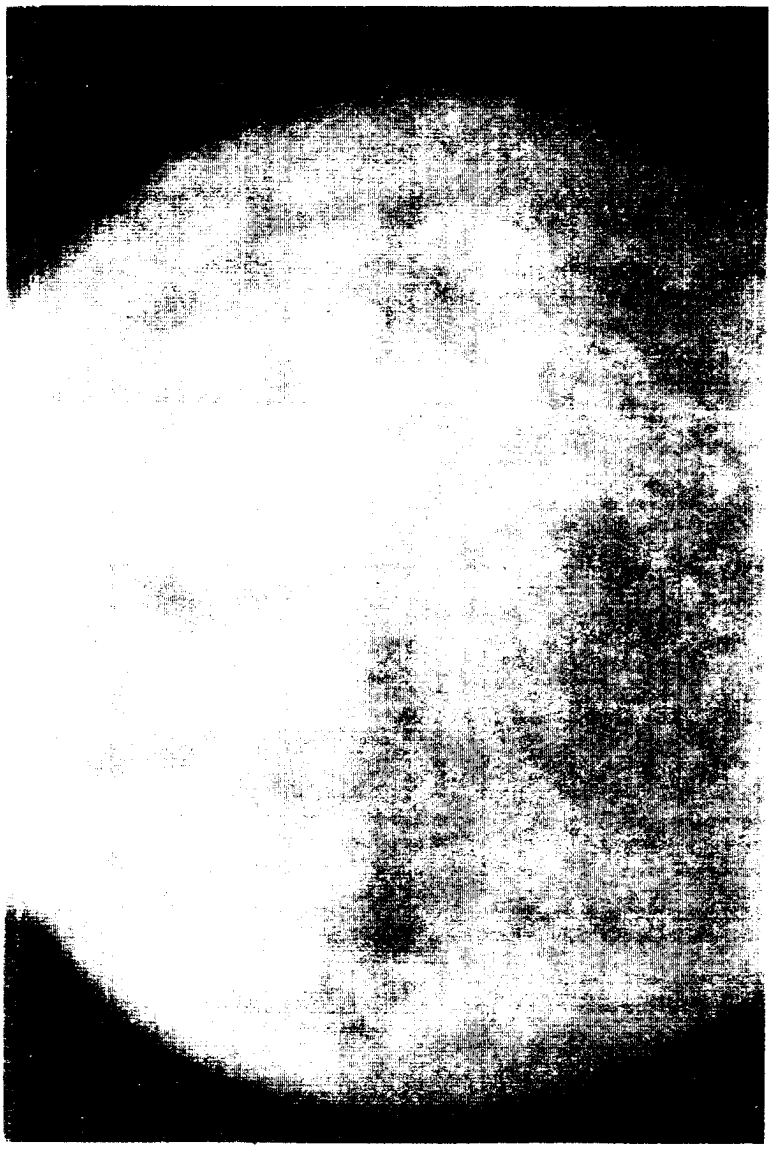


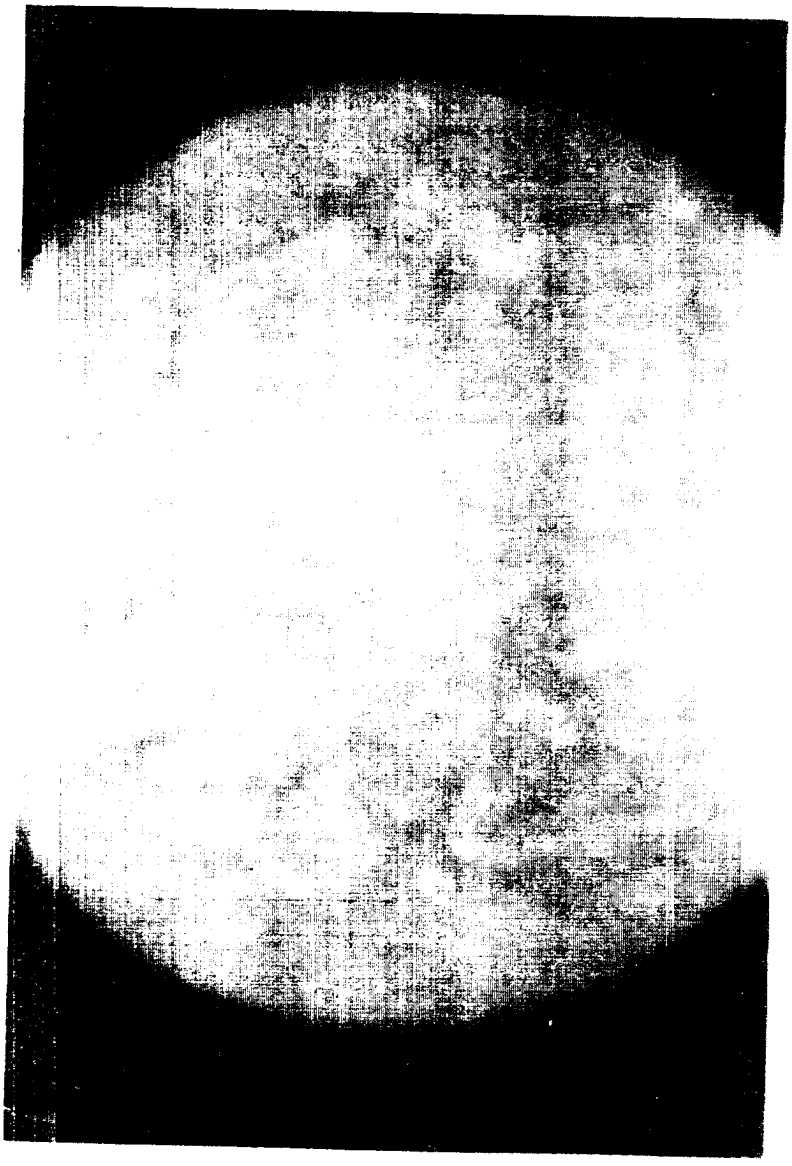
α-CHACONINE



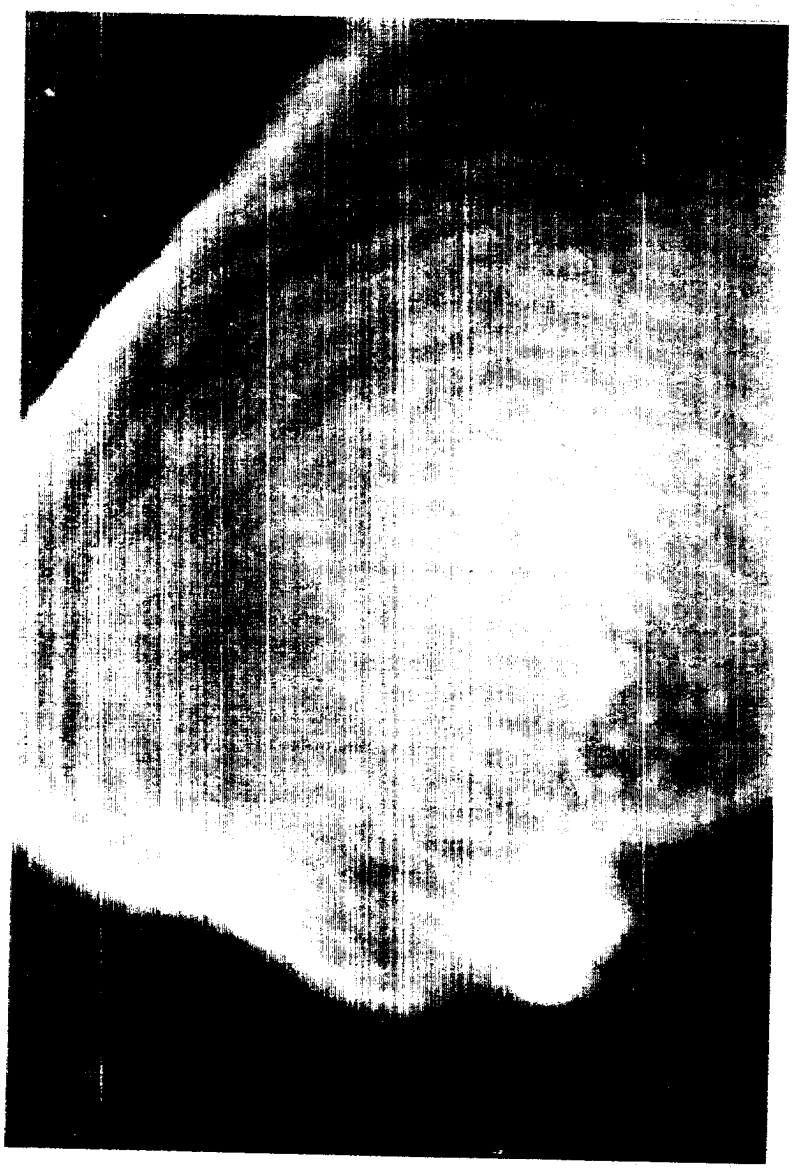






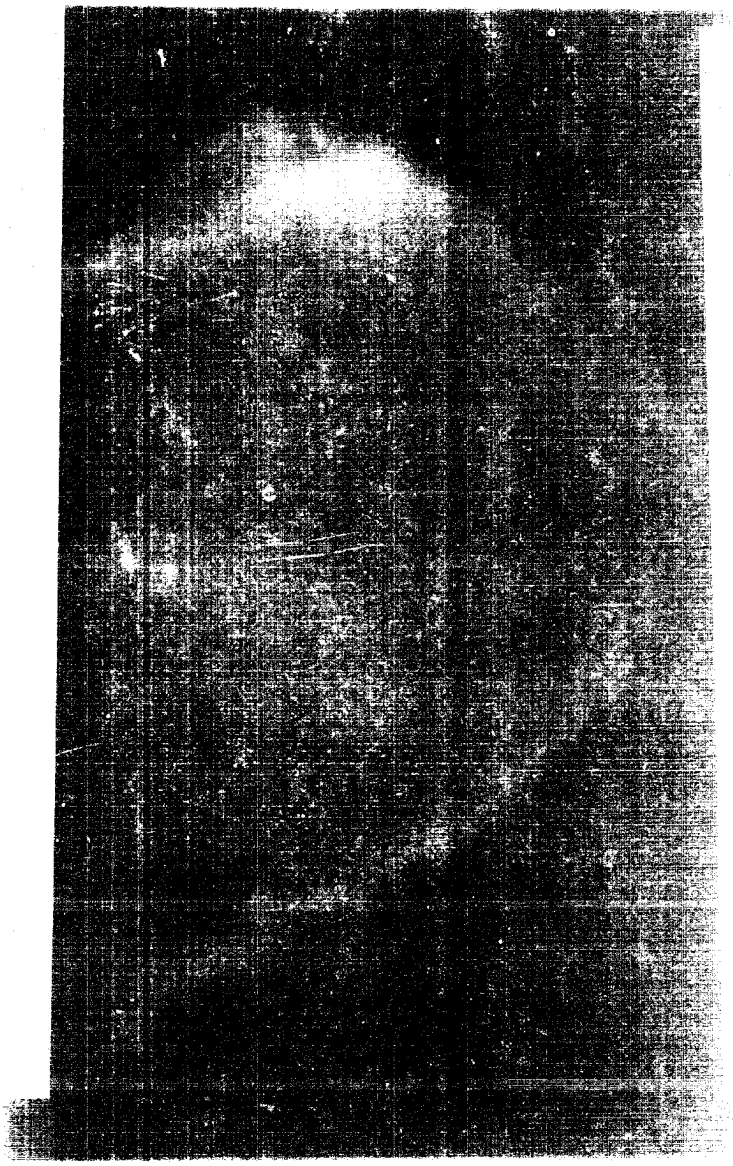


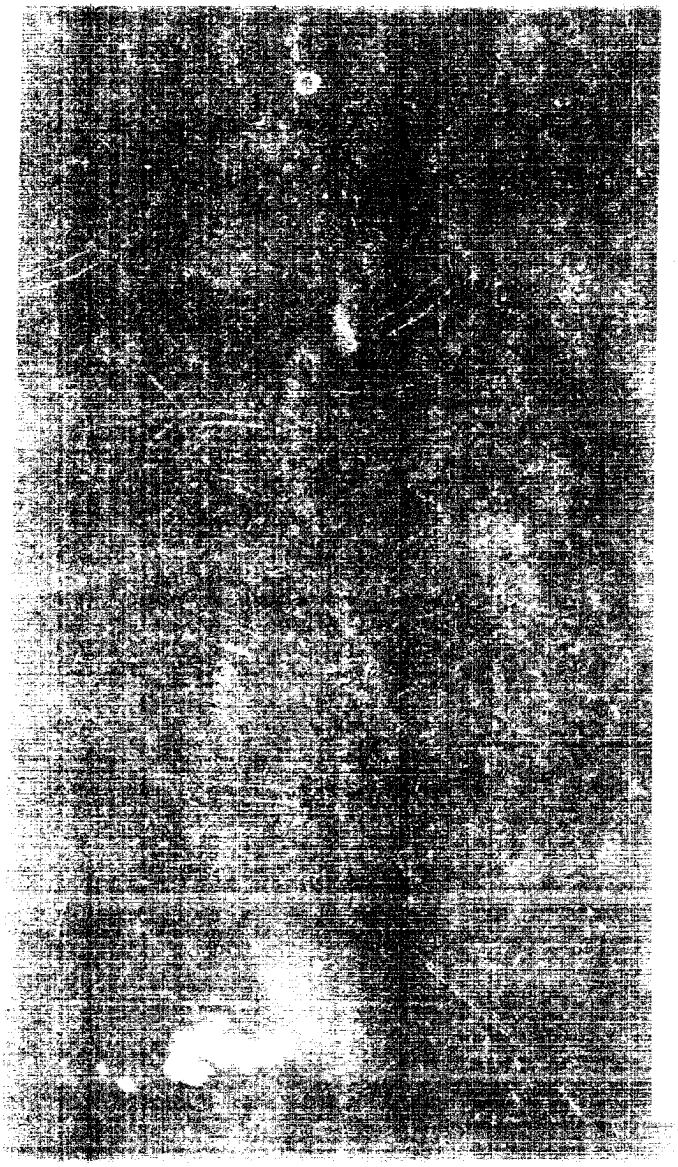
Photomicrograph 7

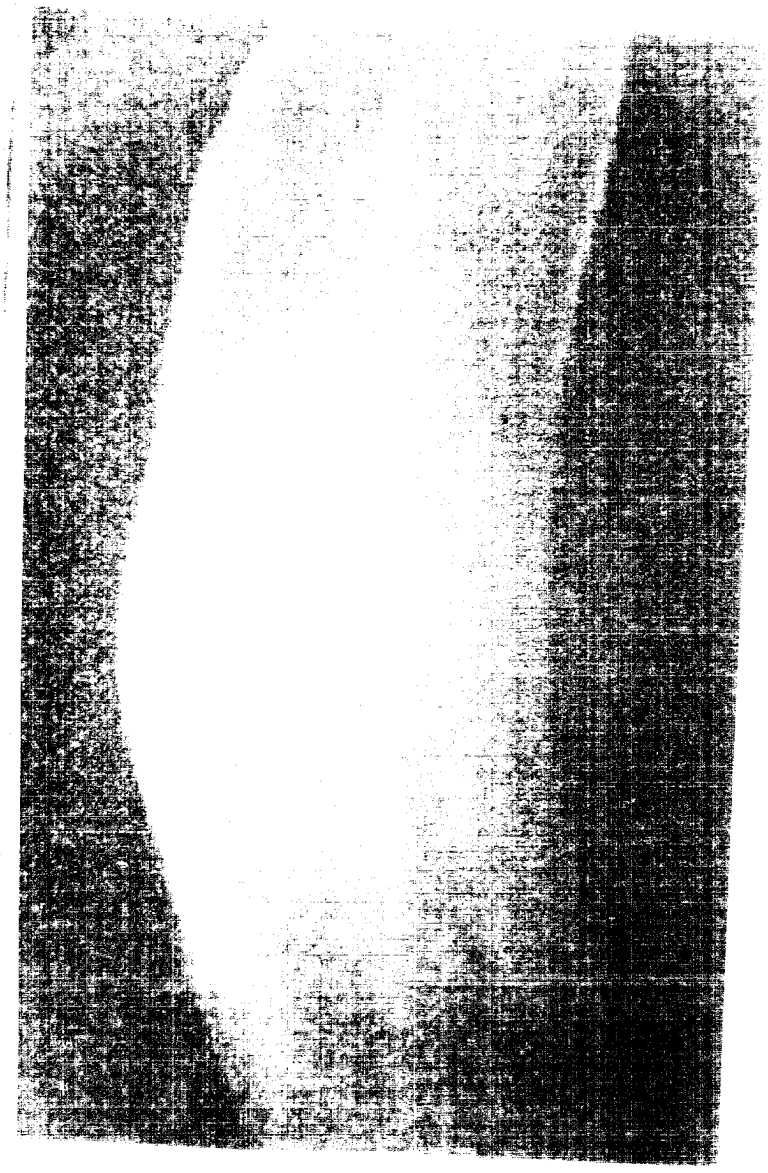


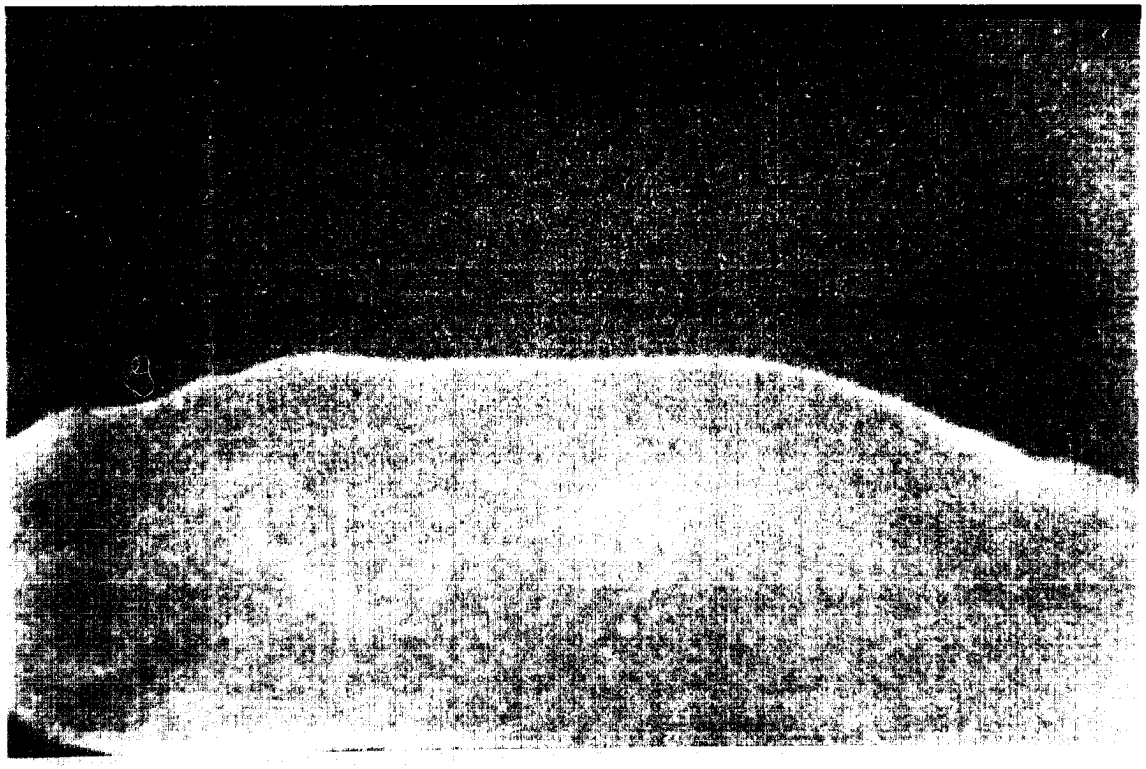
Photomicrograph 8

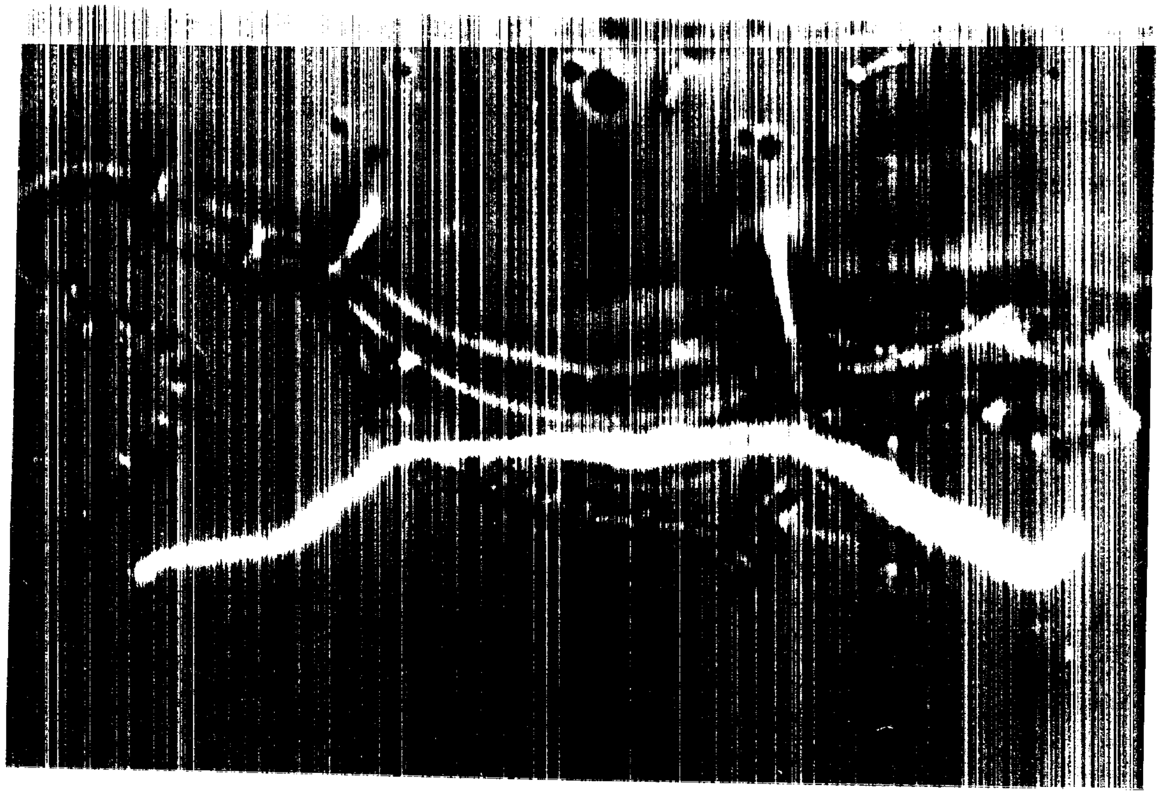
Photomicrograph 4











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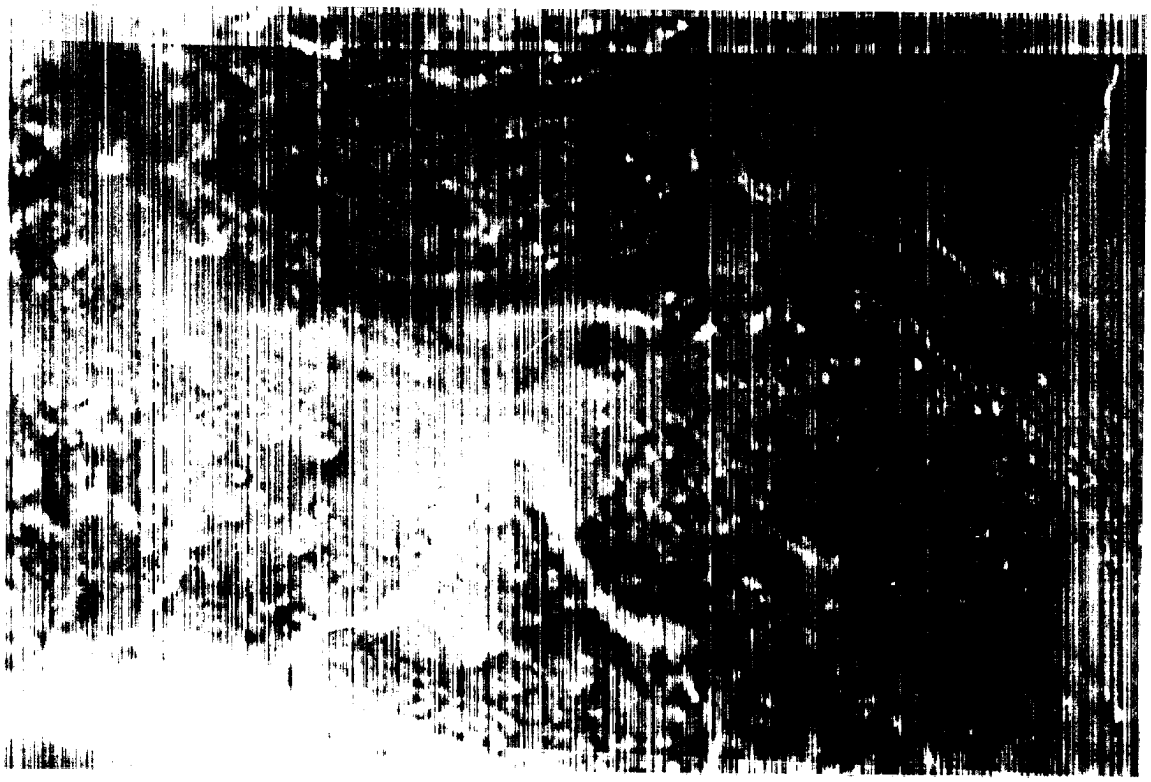
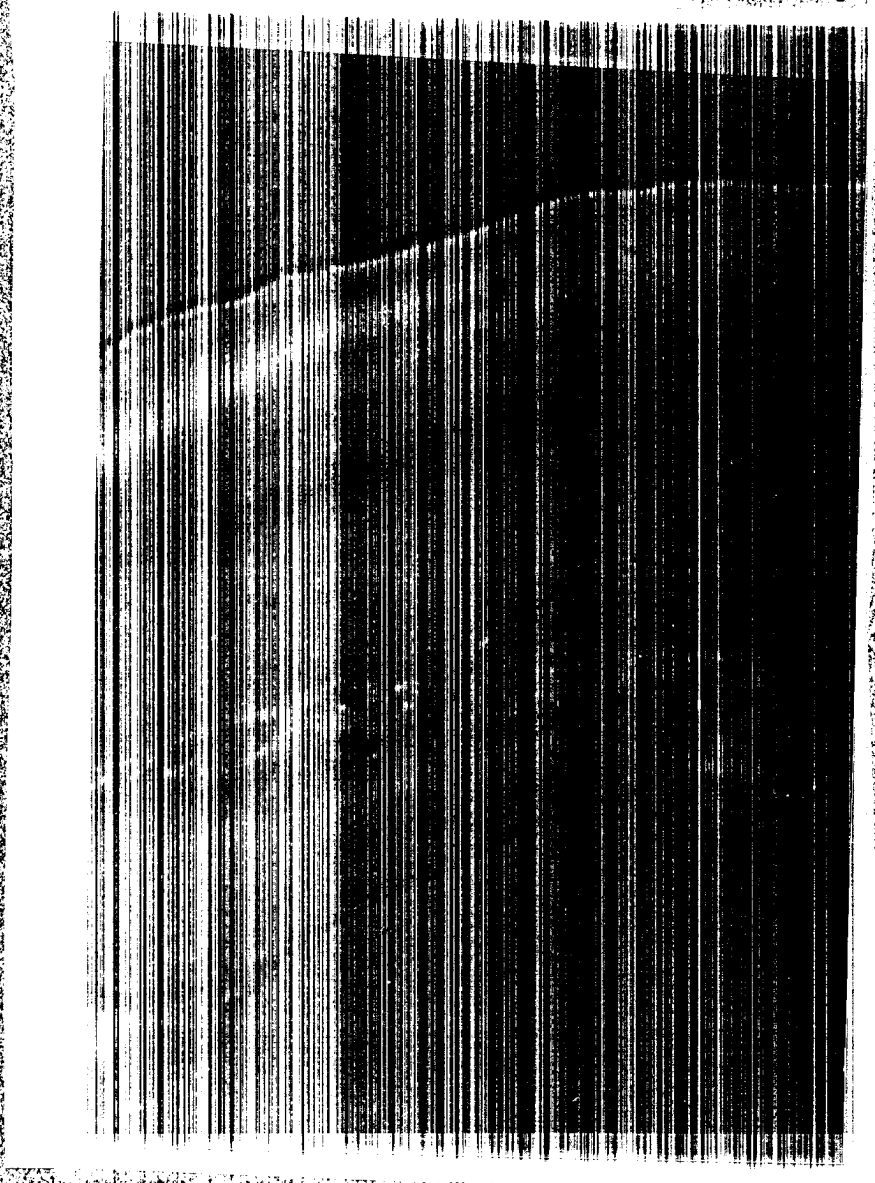
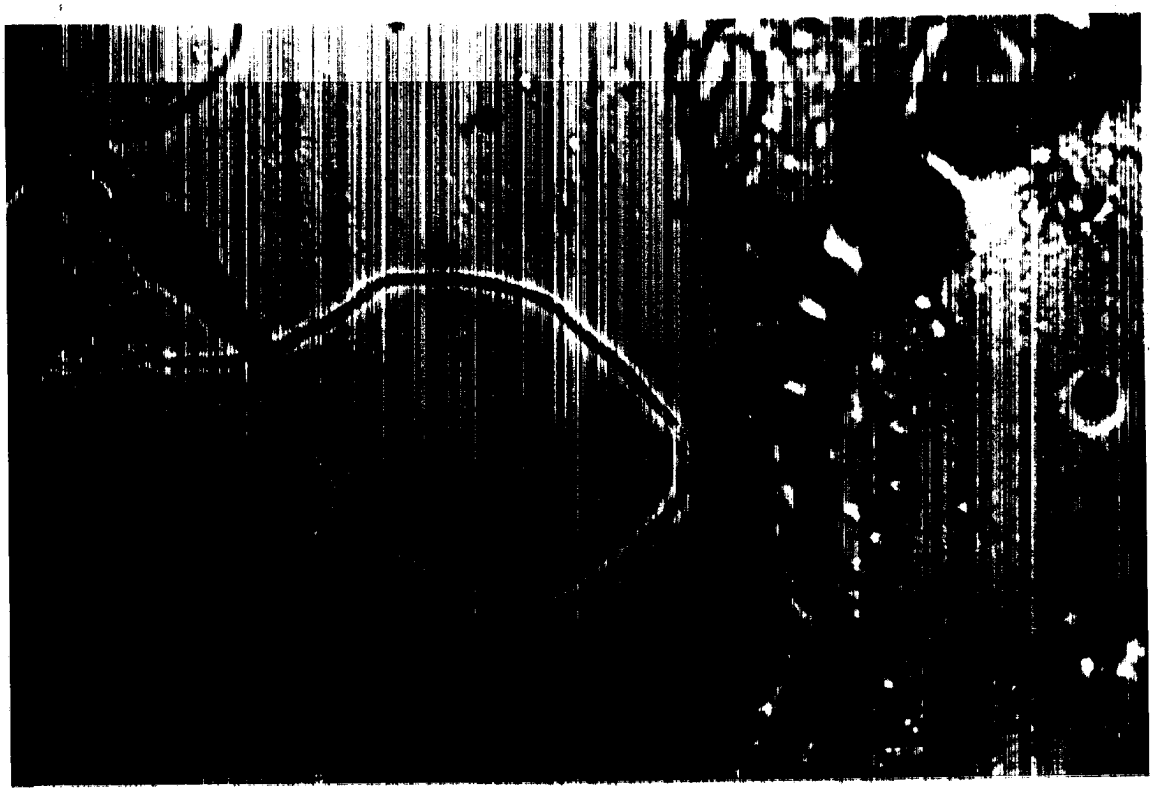
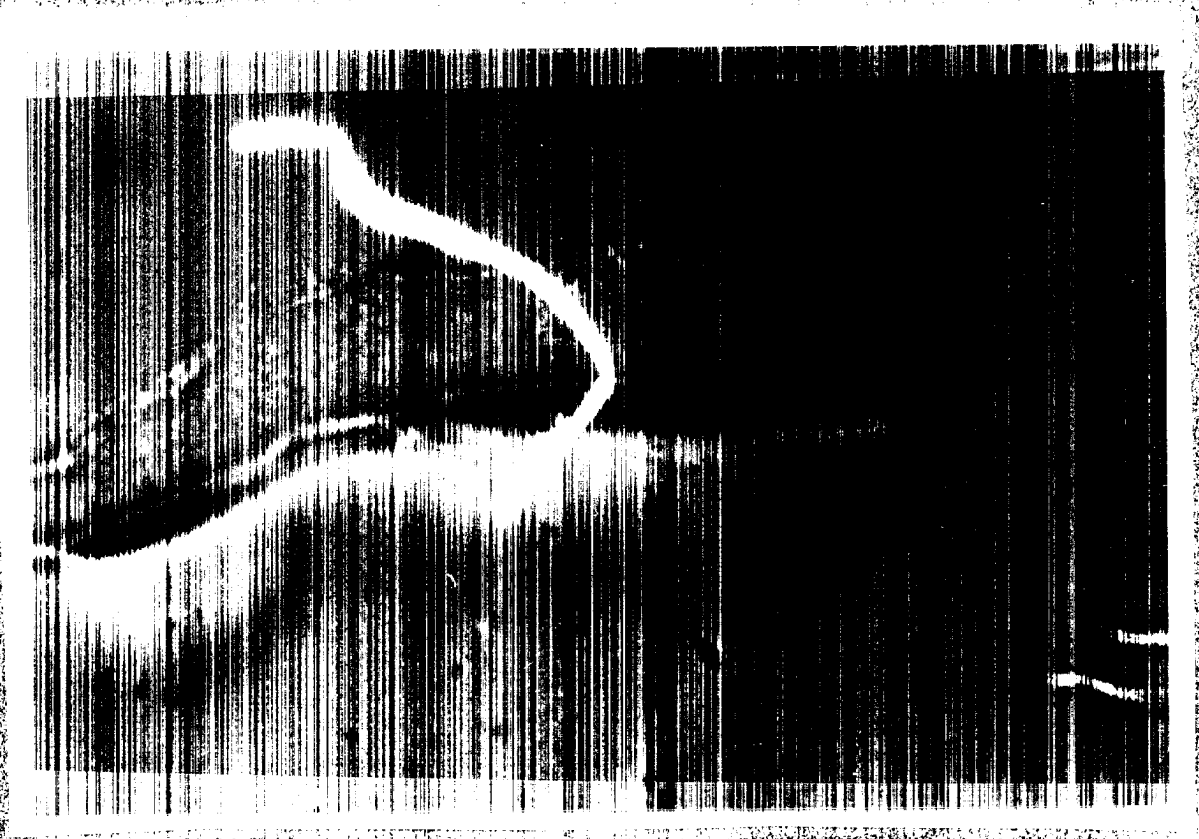


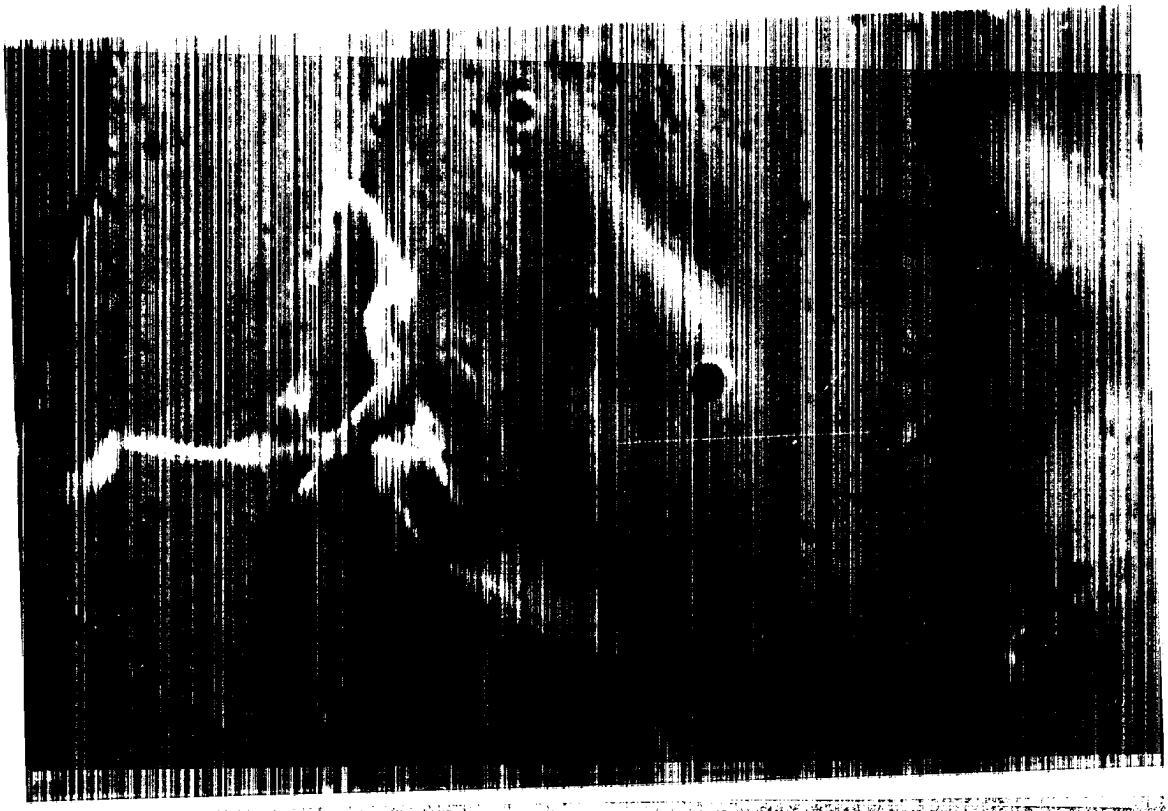


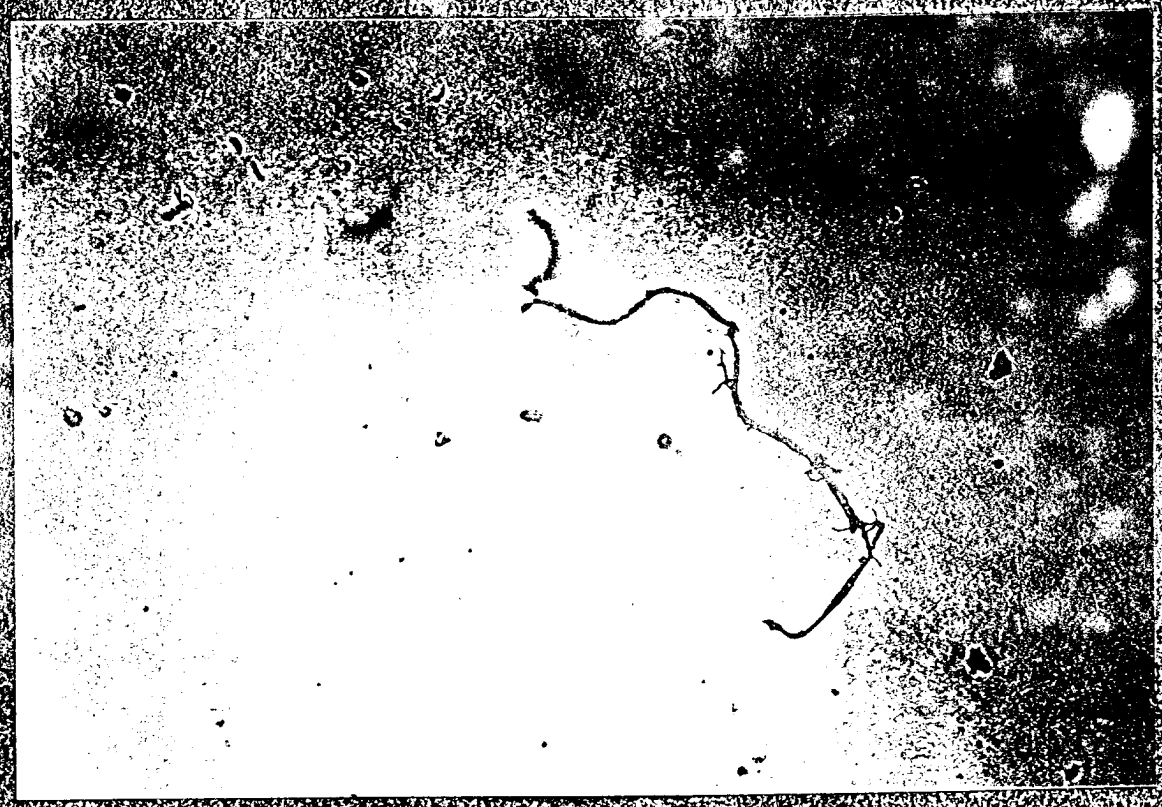
Photograph 28



Photomicrograph 29





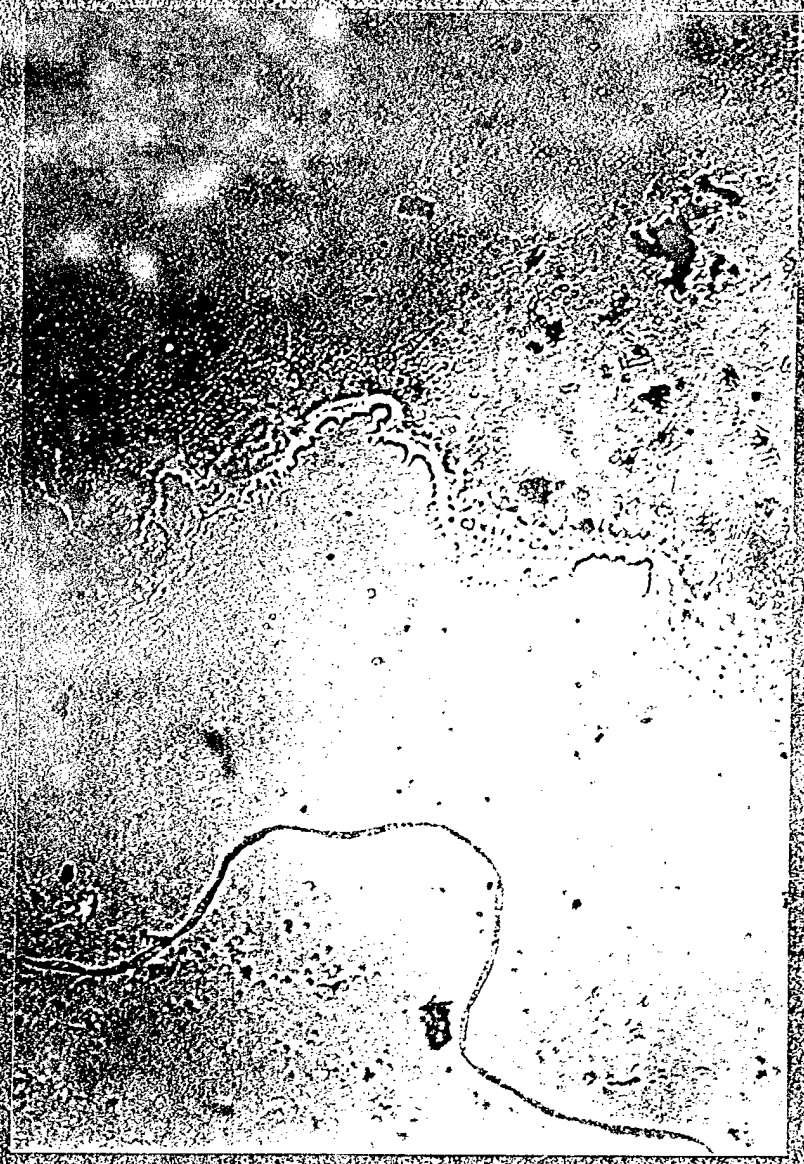


Photomicrograph 32

Photomicrograph 33

Photomicrograph 34

Photomicrograph 35



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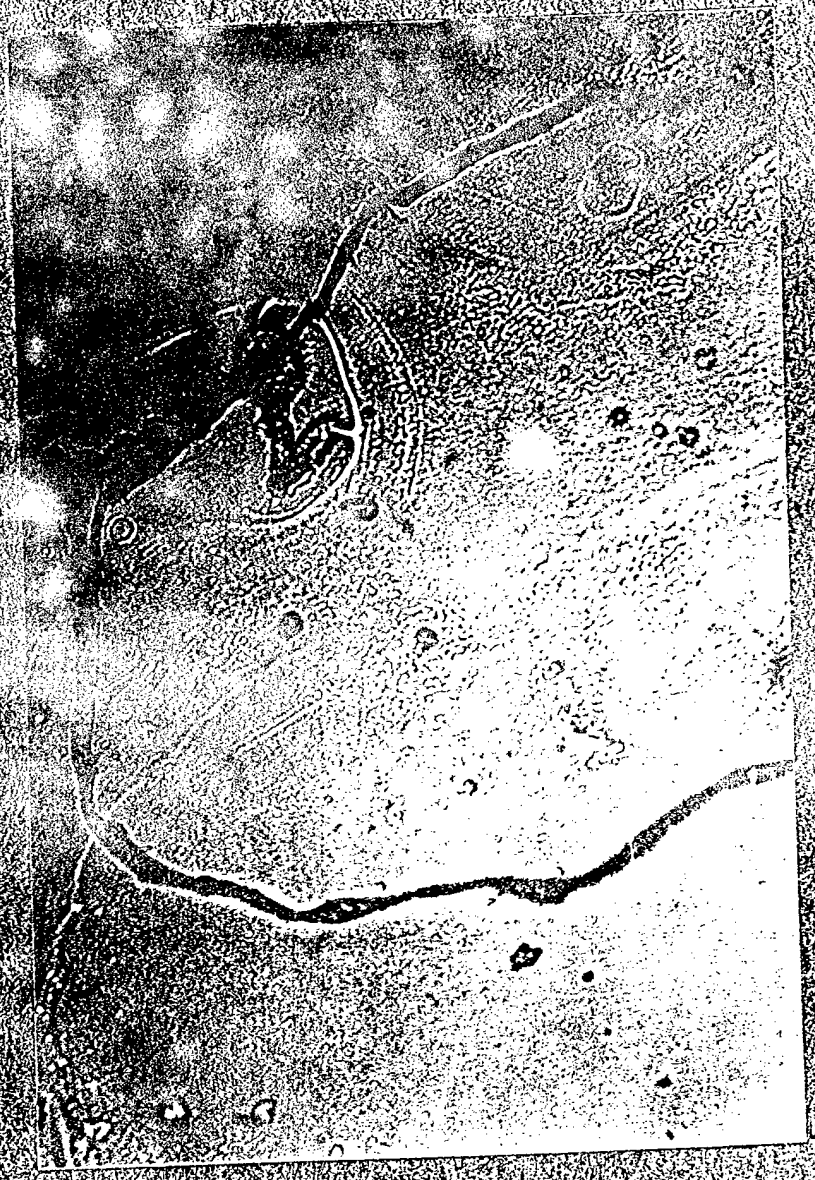

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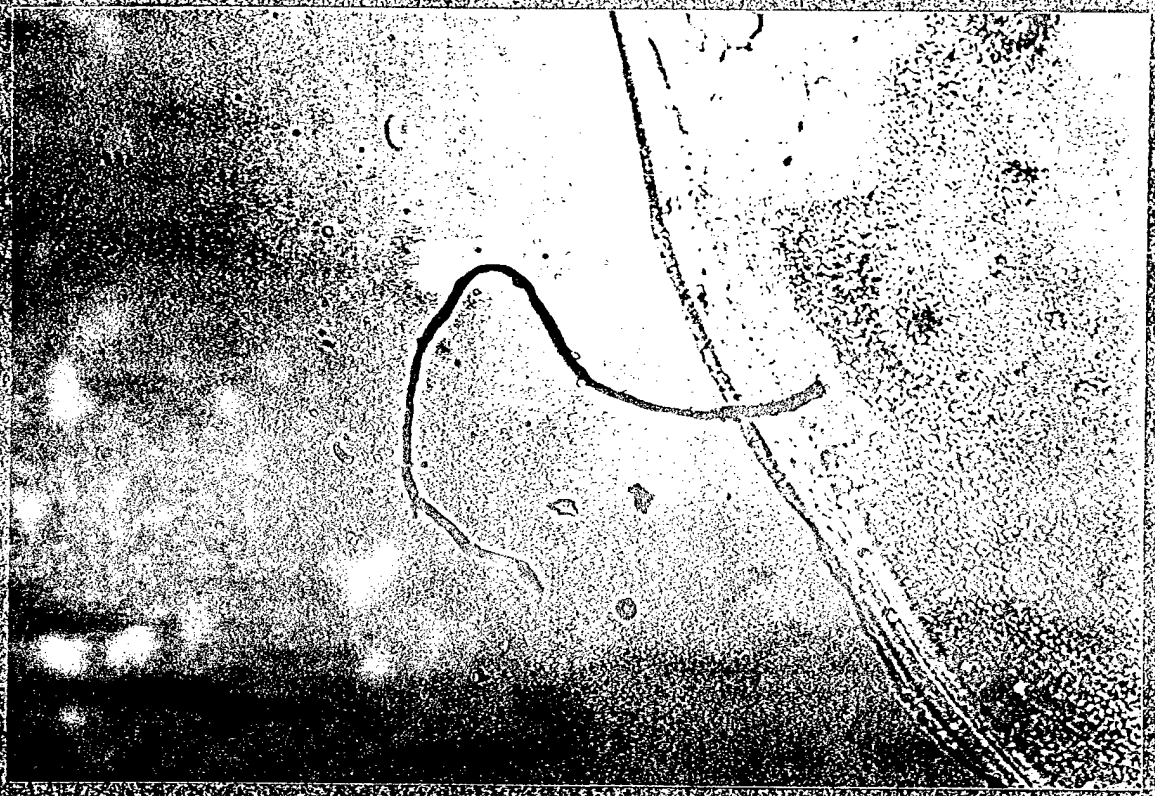

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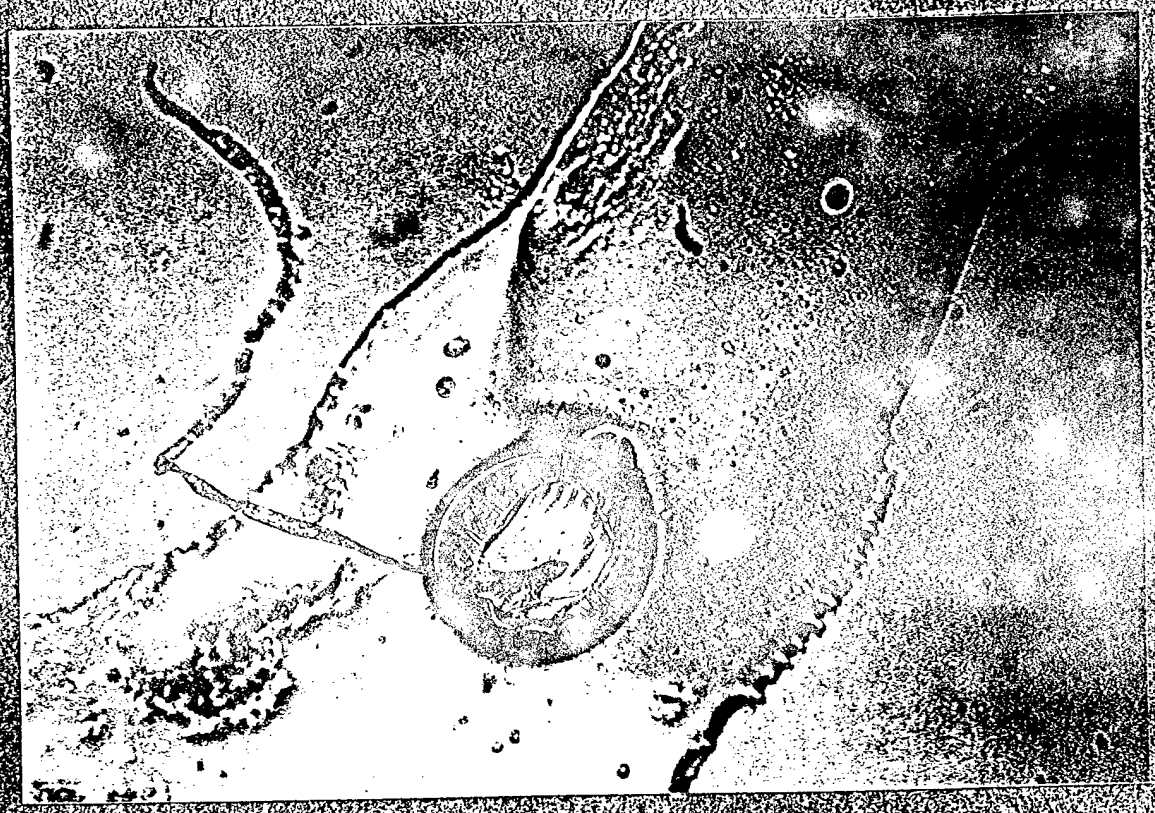

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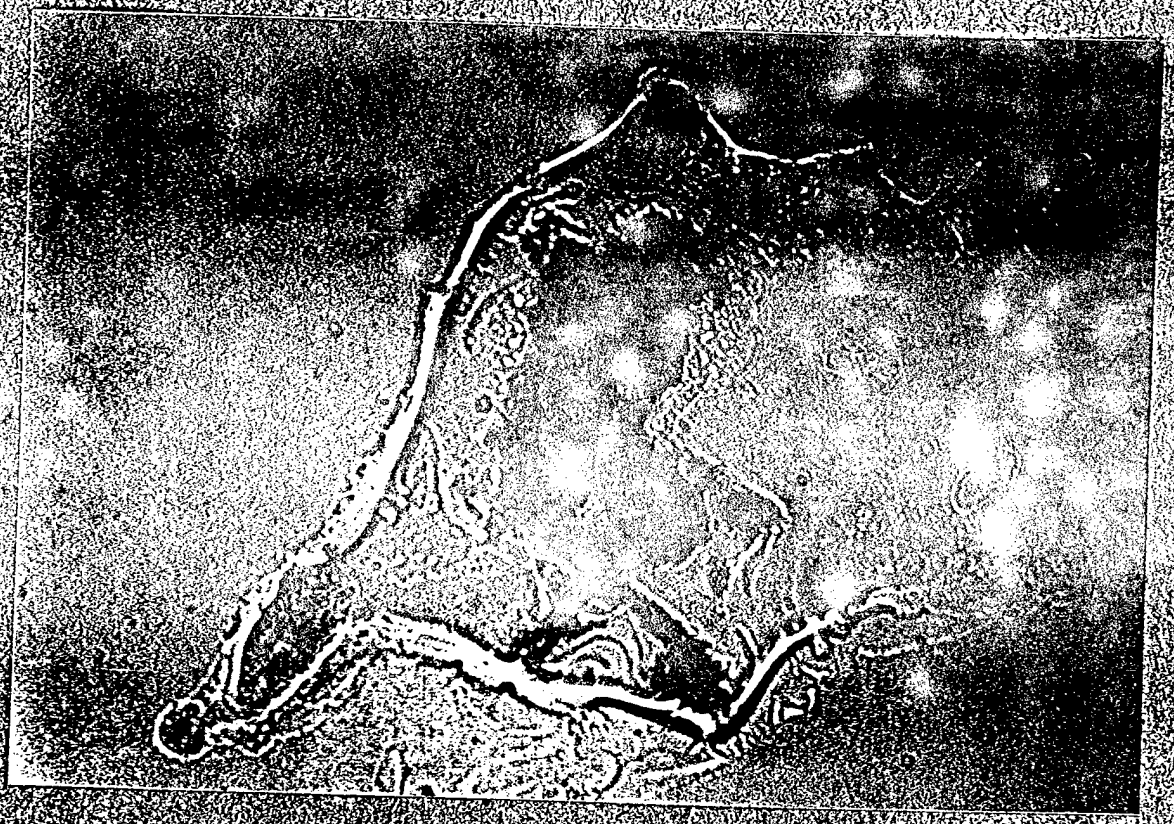
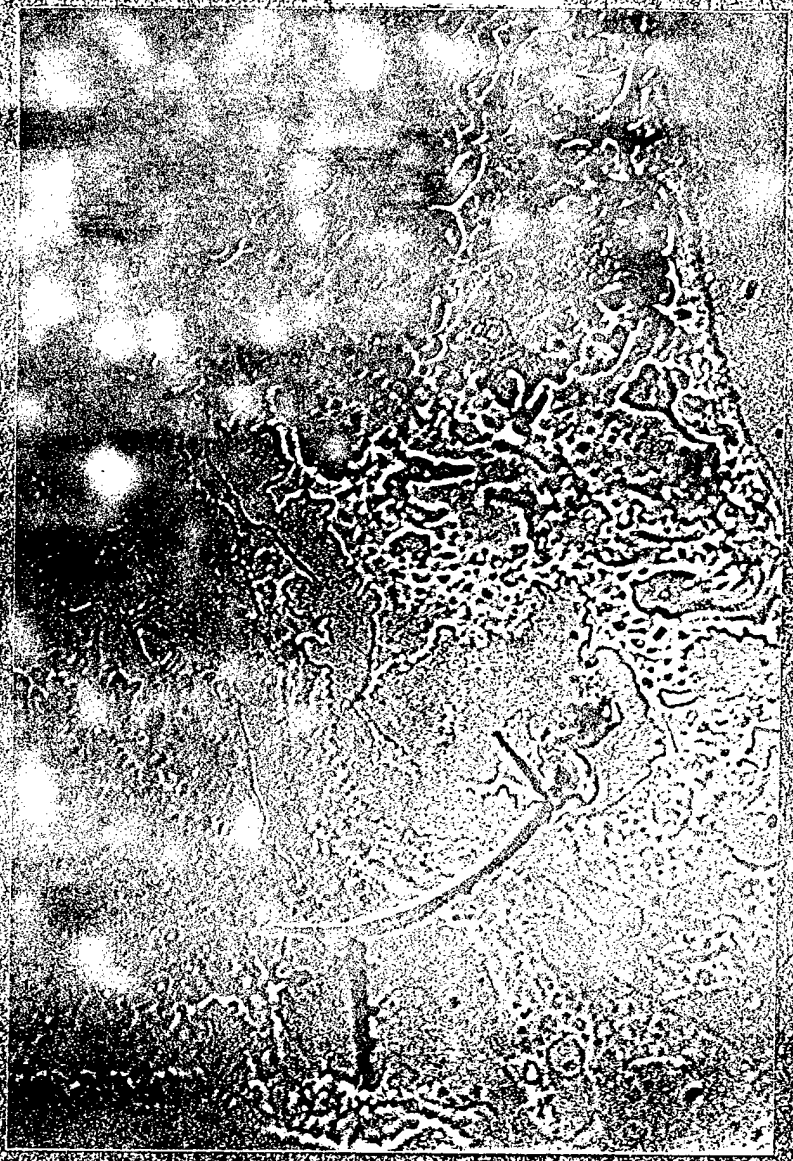


Photomicrograph 40

Photomicrograph 41

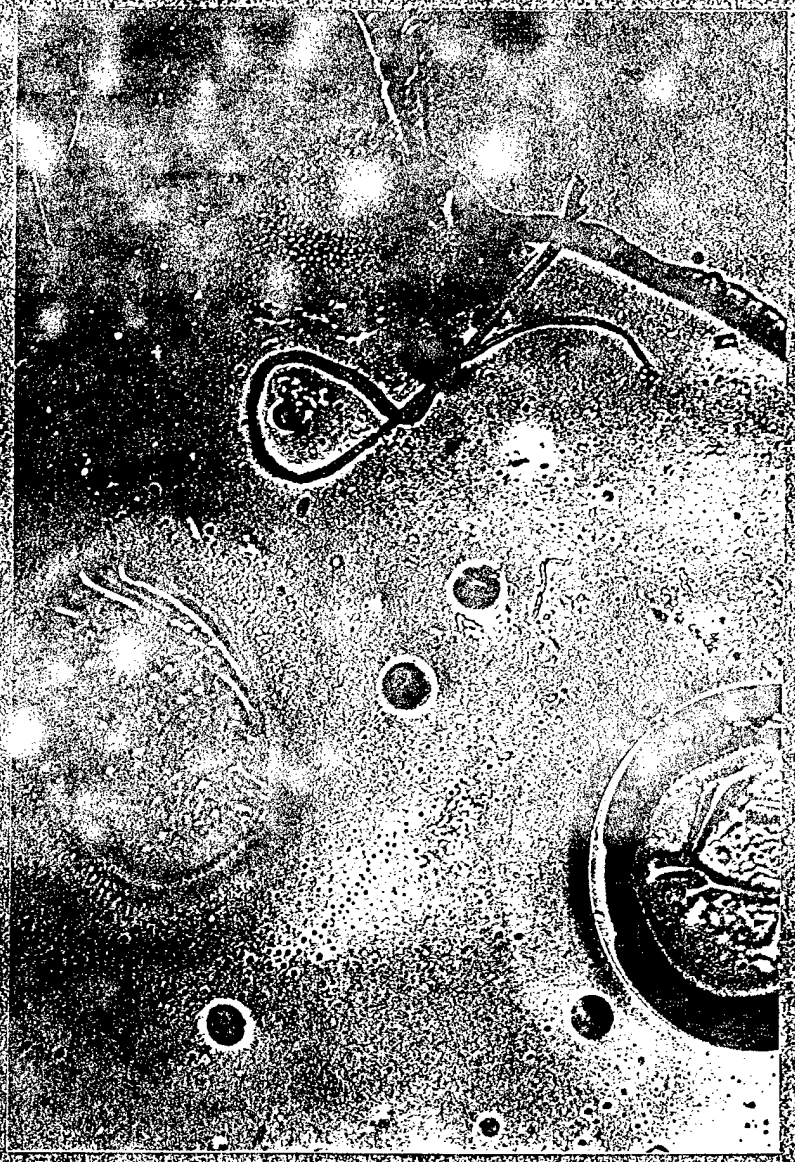


Photomicrograph 42

Photomicrograph 43

Photograph 44

Photomicrograph 45



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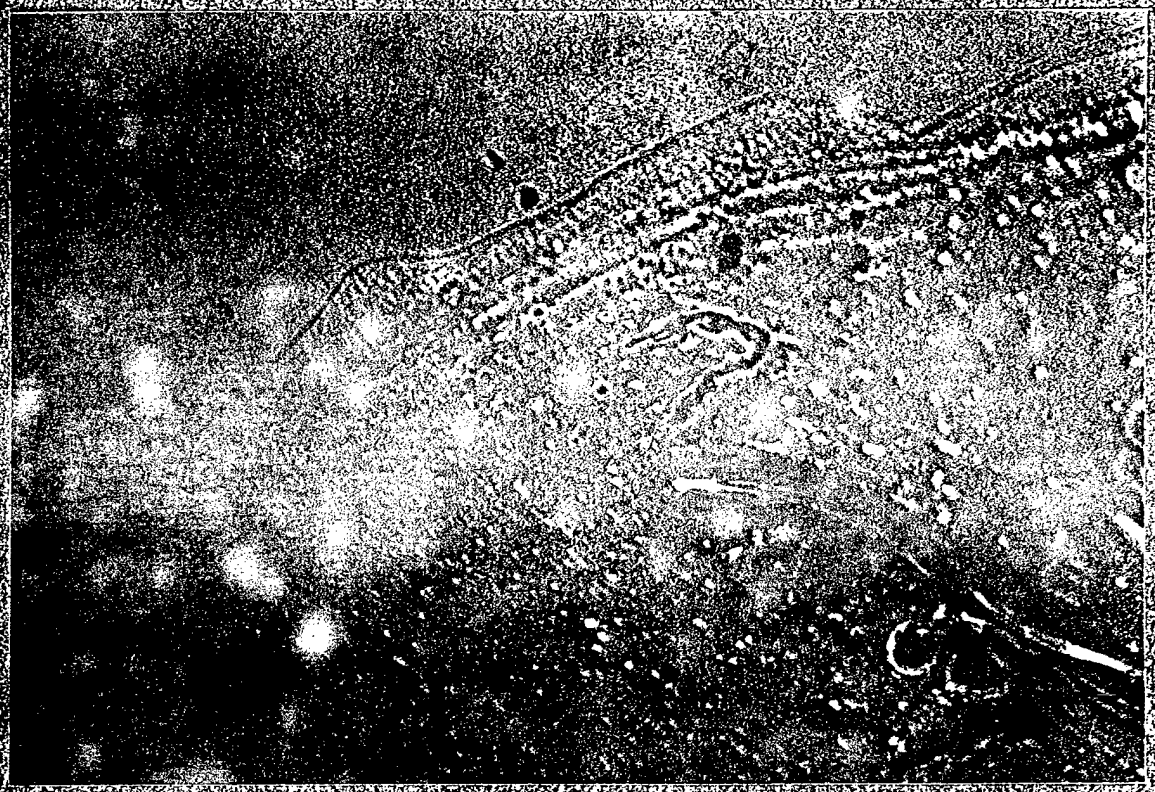
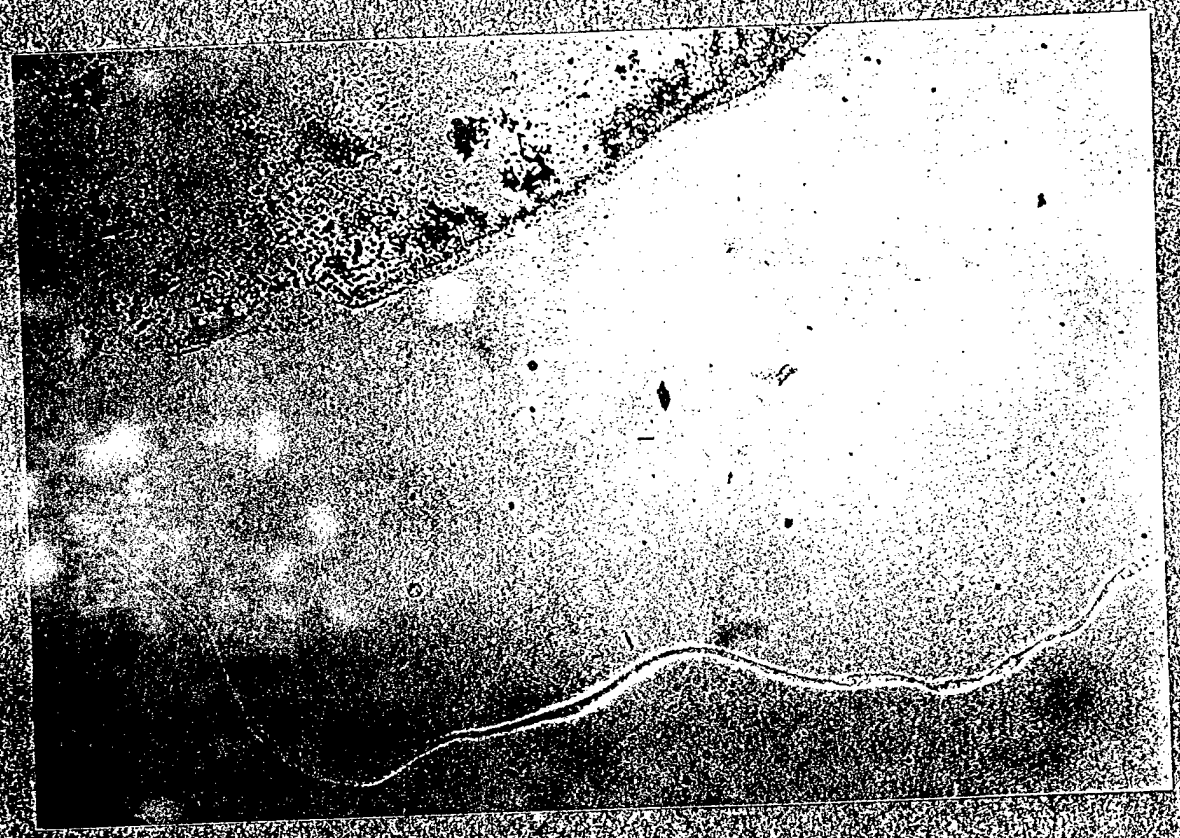
Photomicrograph 47

Photomicrograph 48

Photomicrograph 49



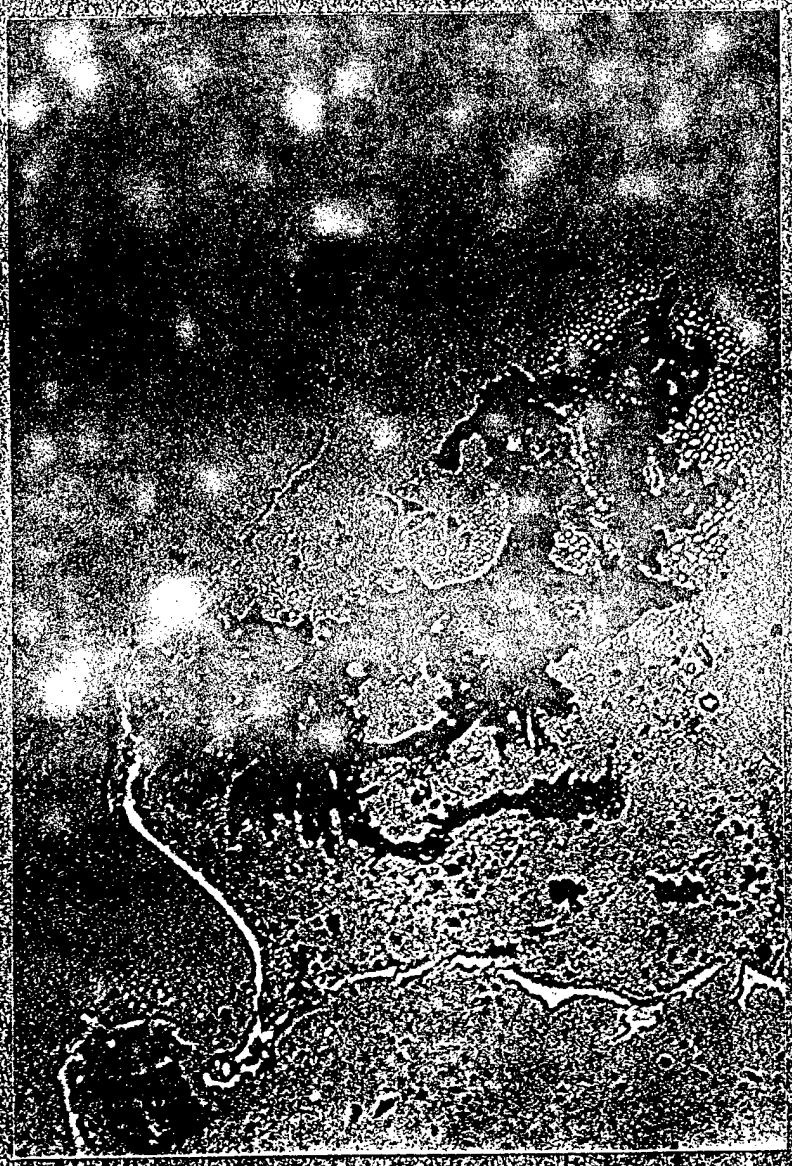
Photomicrograph 50



Photomicrograph 51

Photomicrograph 52

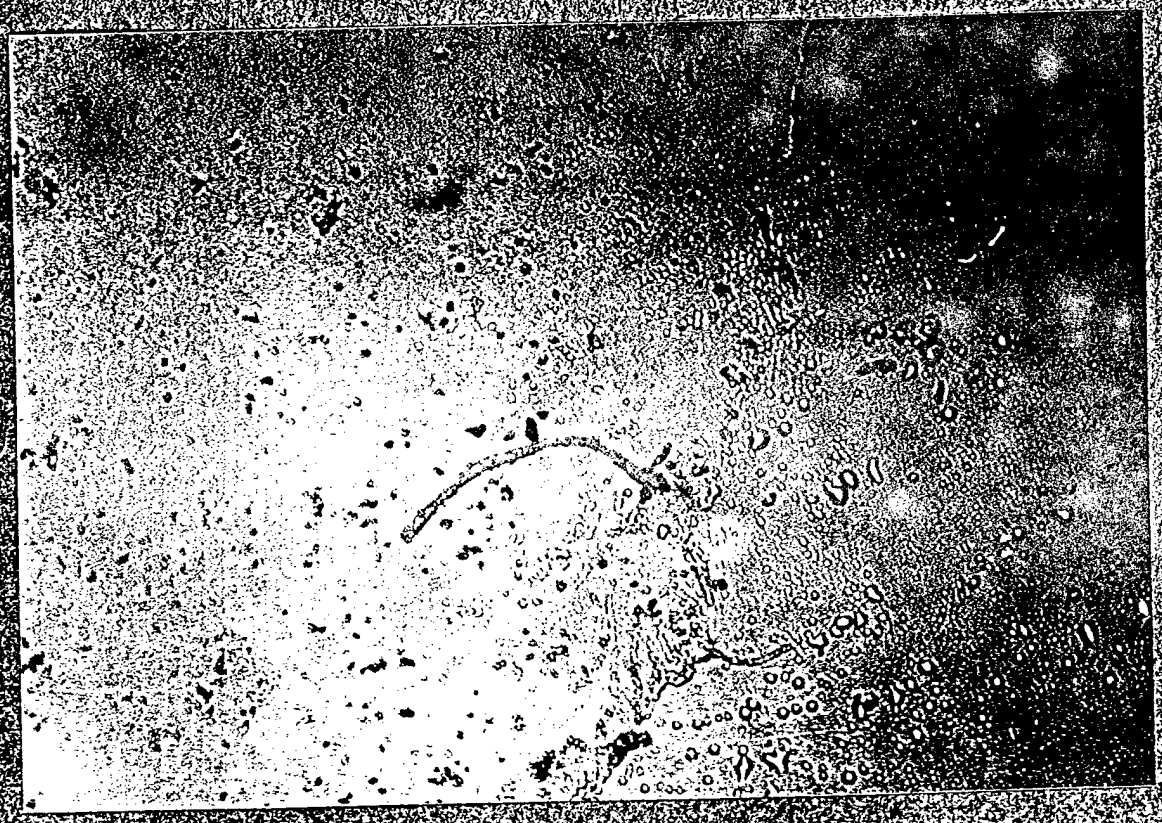
Photomicrograph 53



Photomicrograph 54

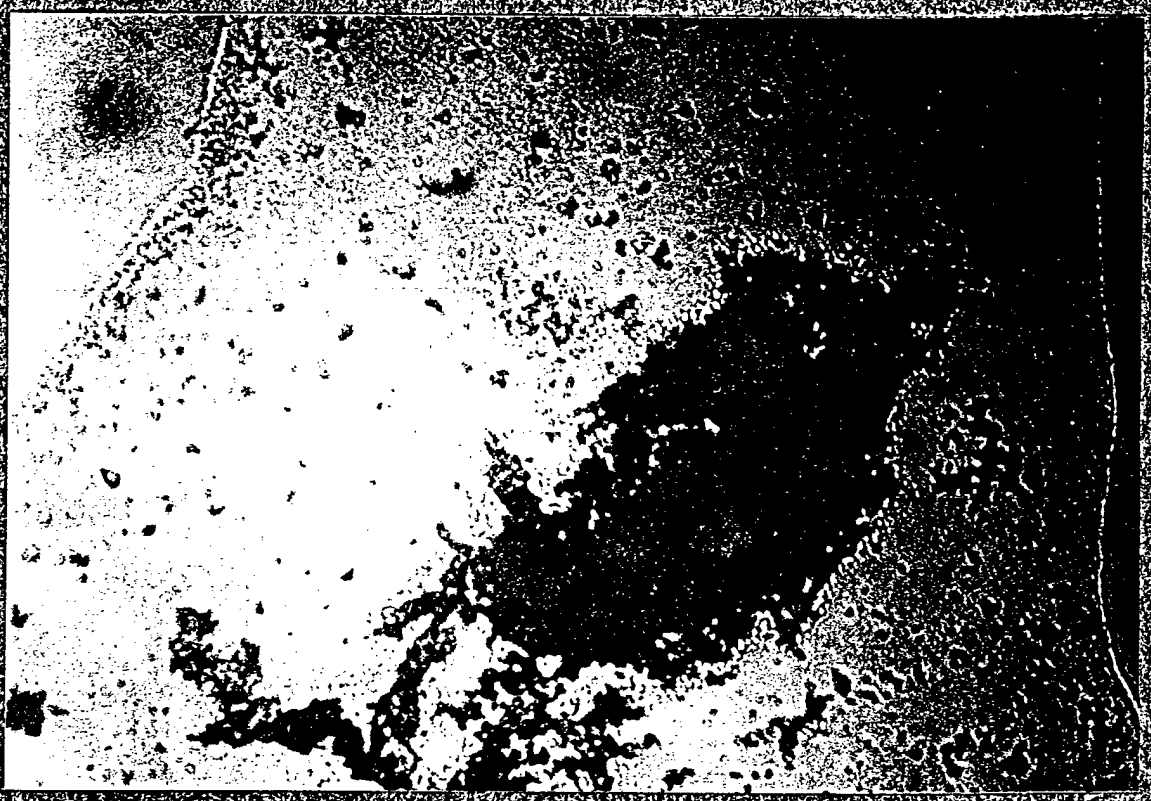
Photomicrograph 55

Photomicrograph 56



Photomicrograph 57

Photomicrograph 58



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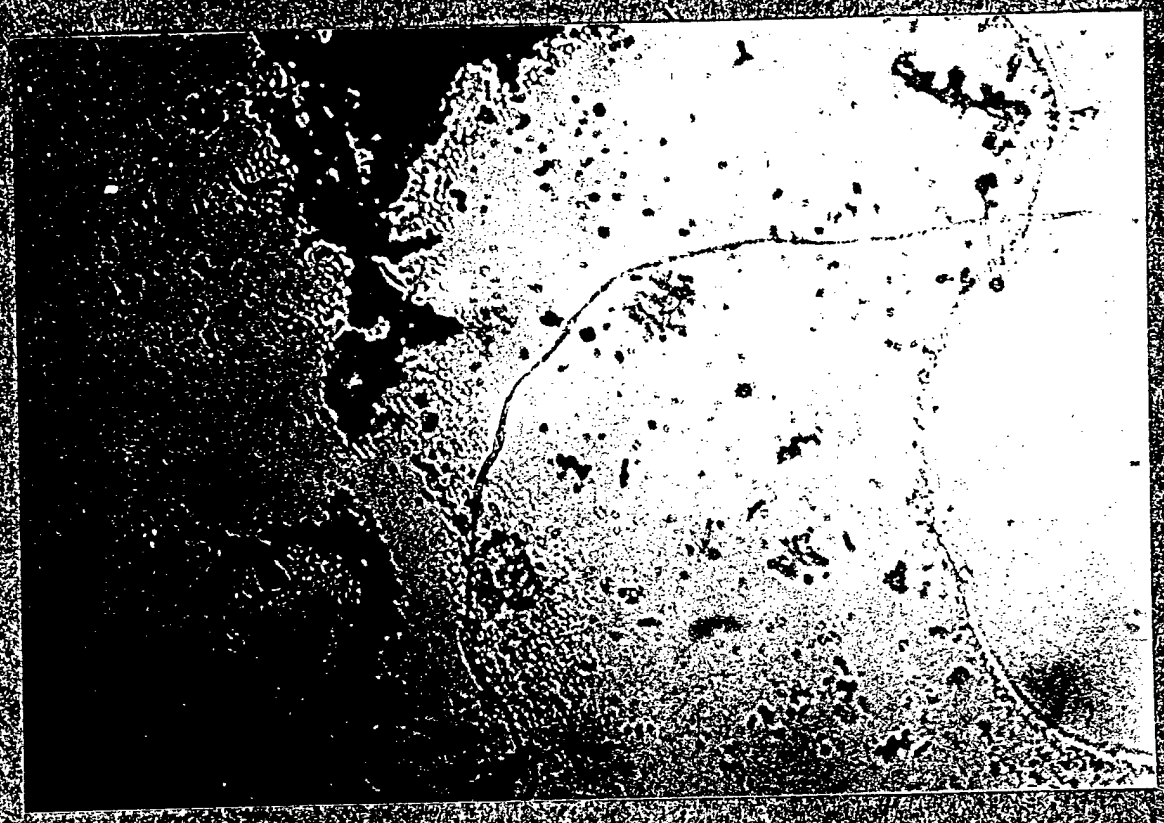
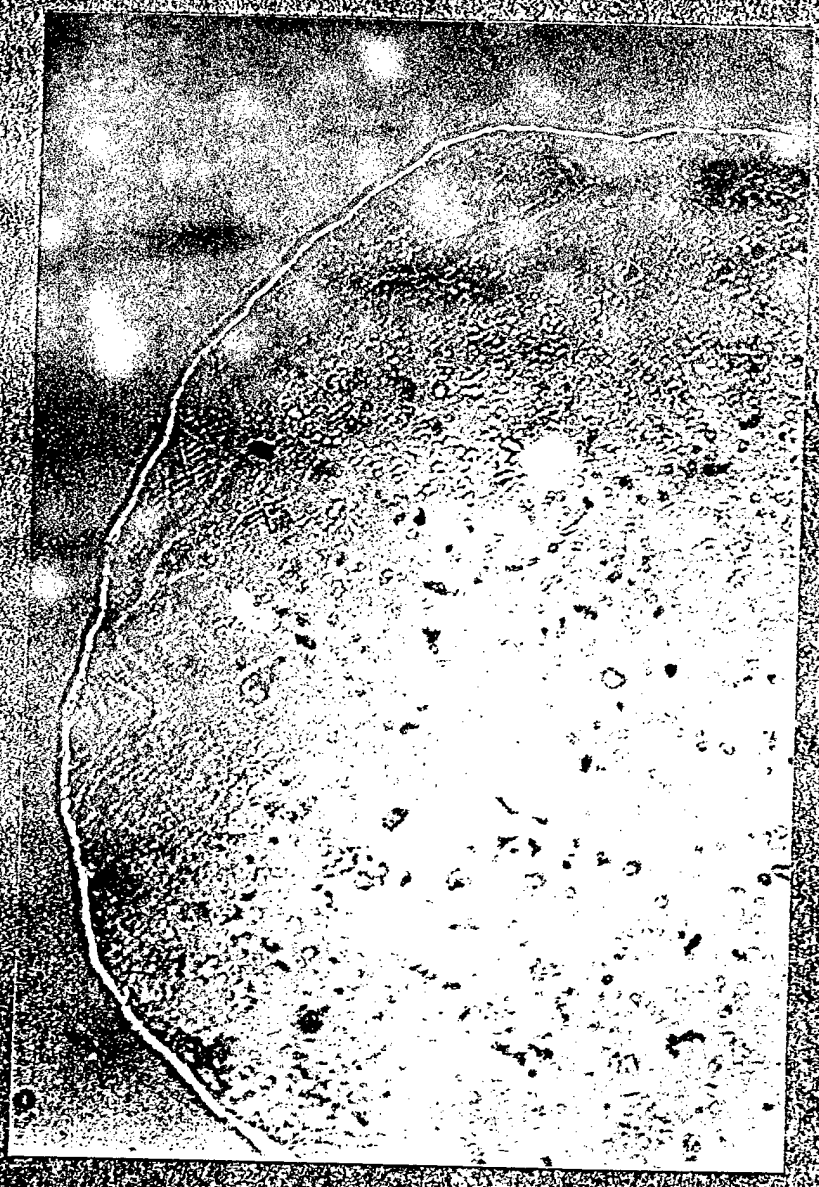


Photomicrograph 60

Photomicrograph 61



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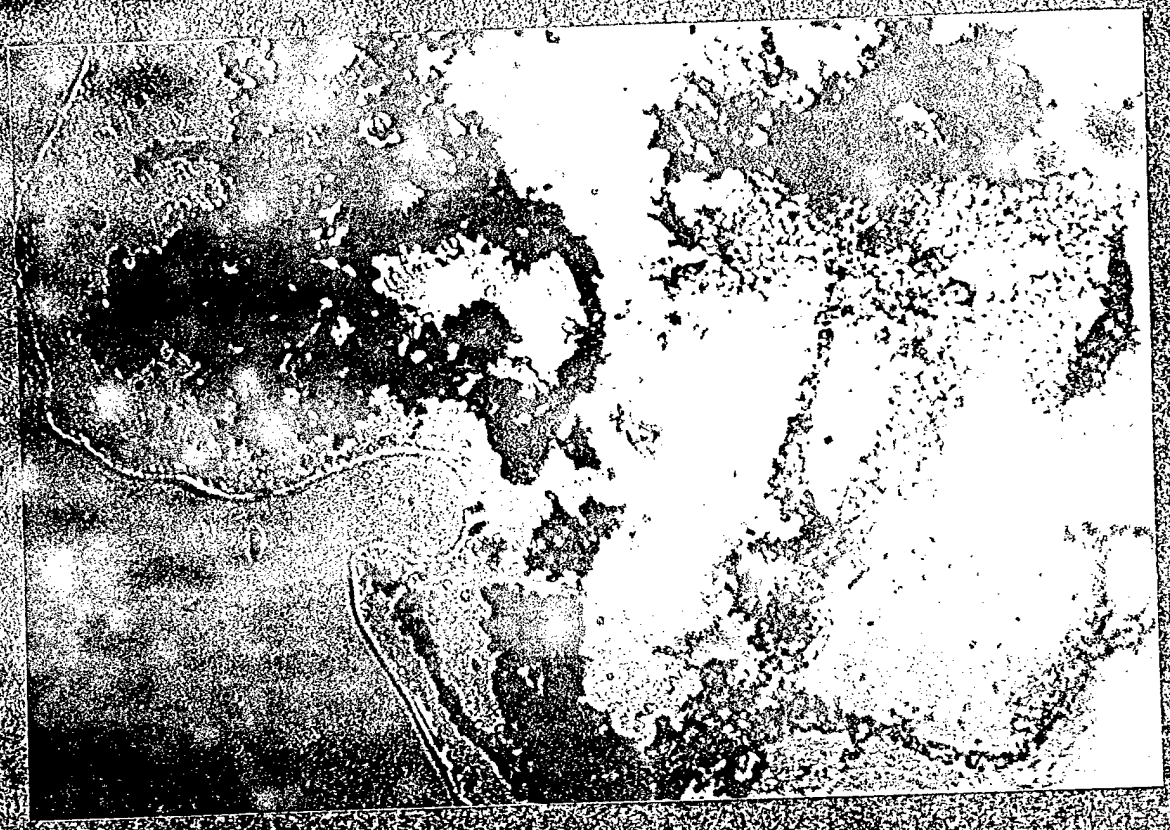
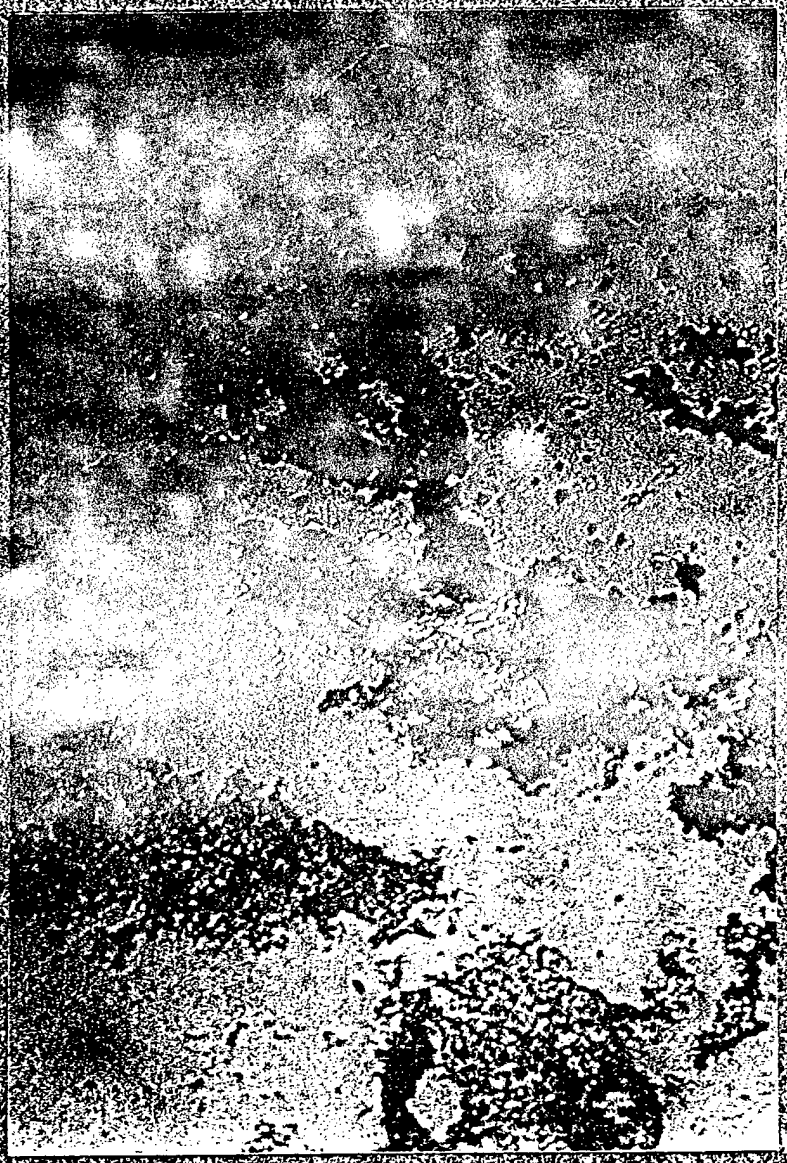


Photomicrograph 63

Photomicrograph 64

Photomicrograph 65



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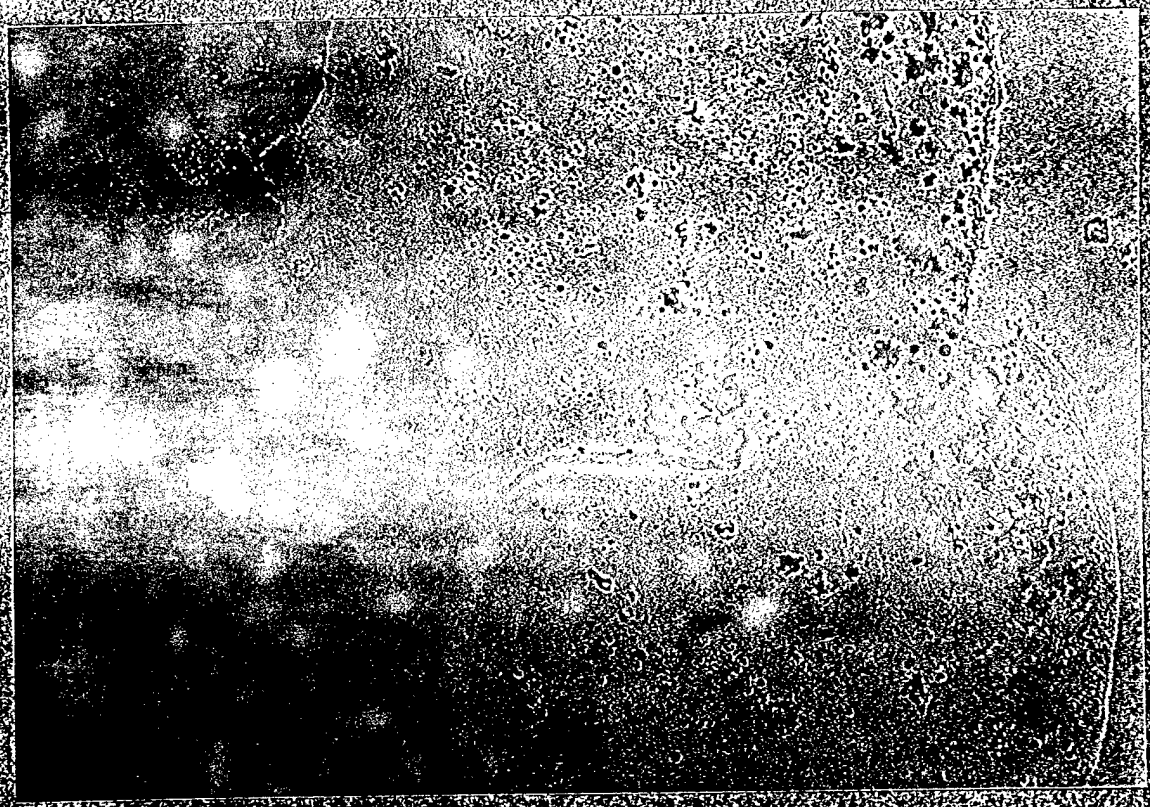

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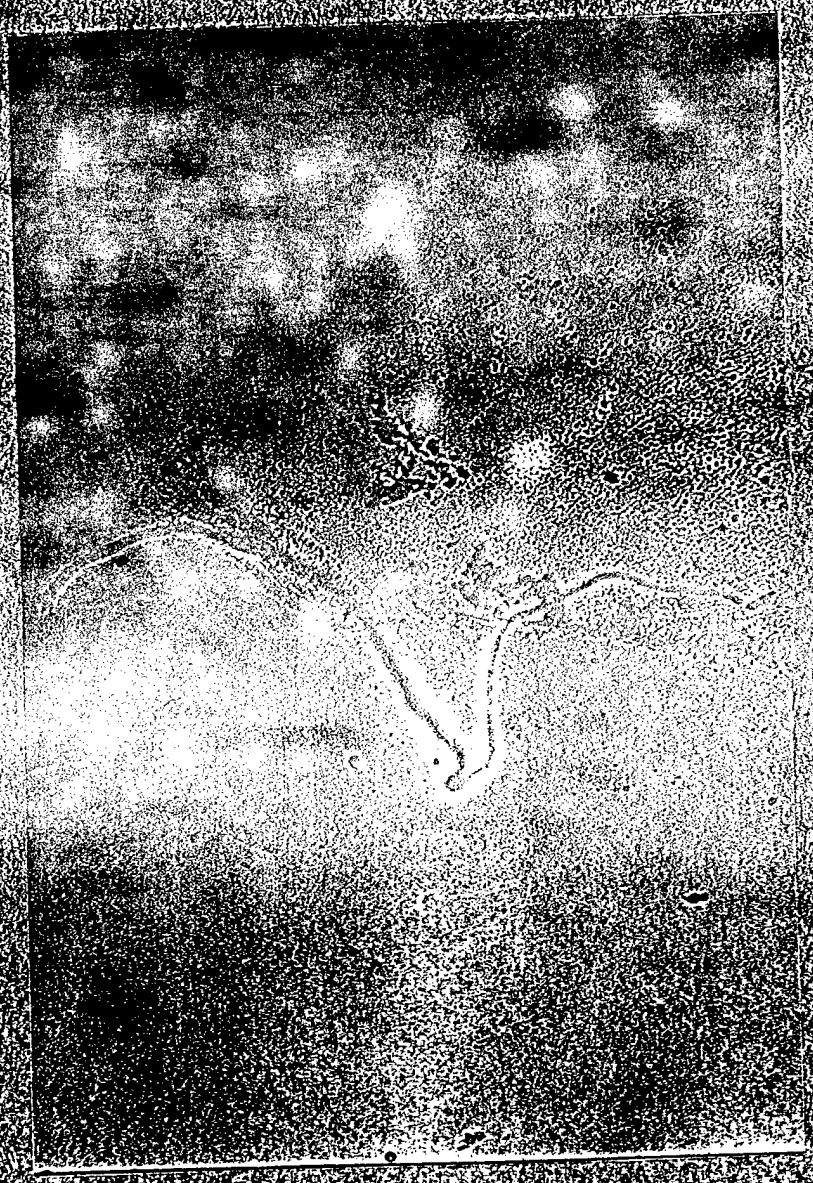
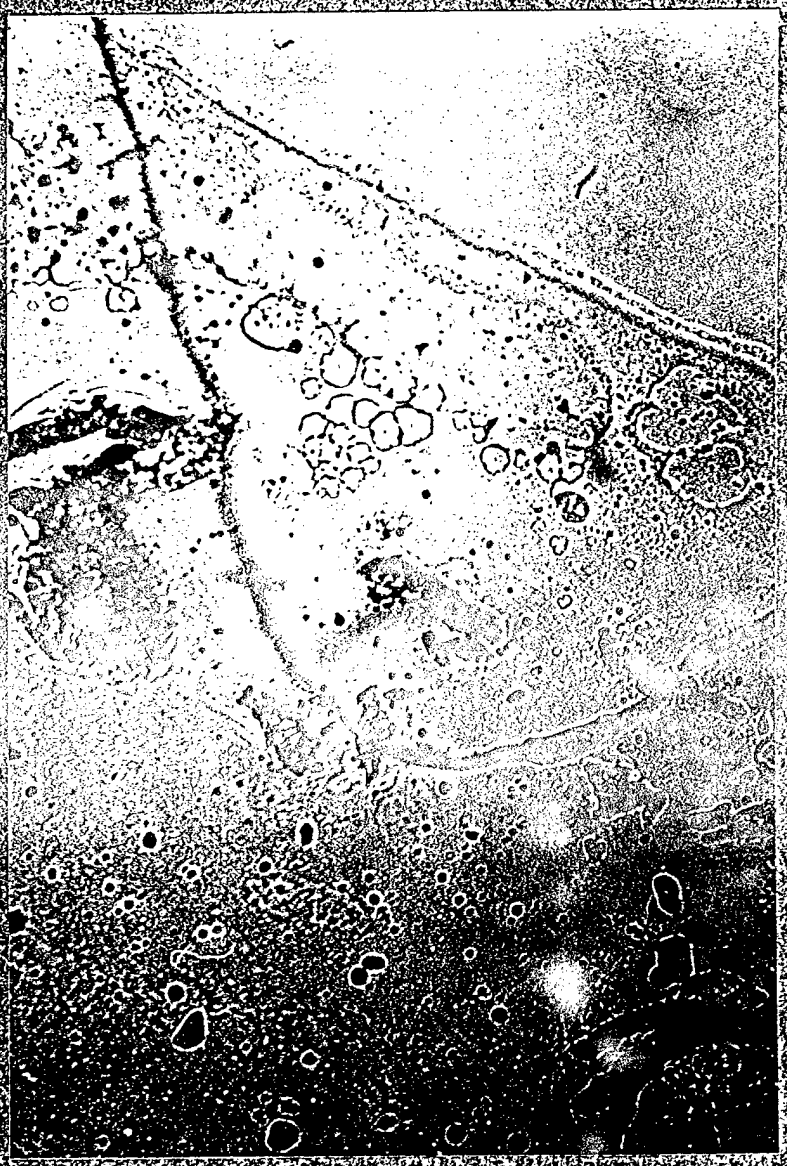
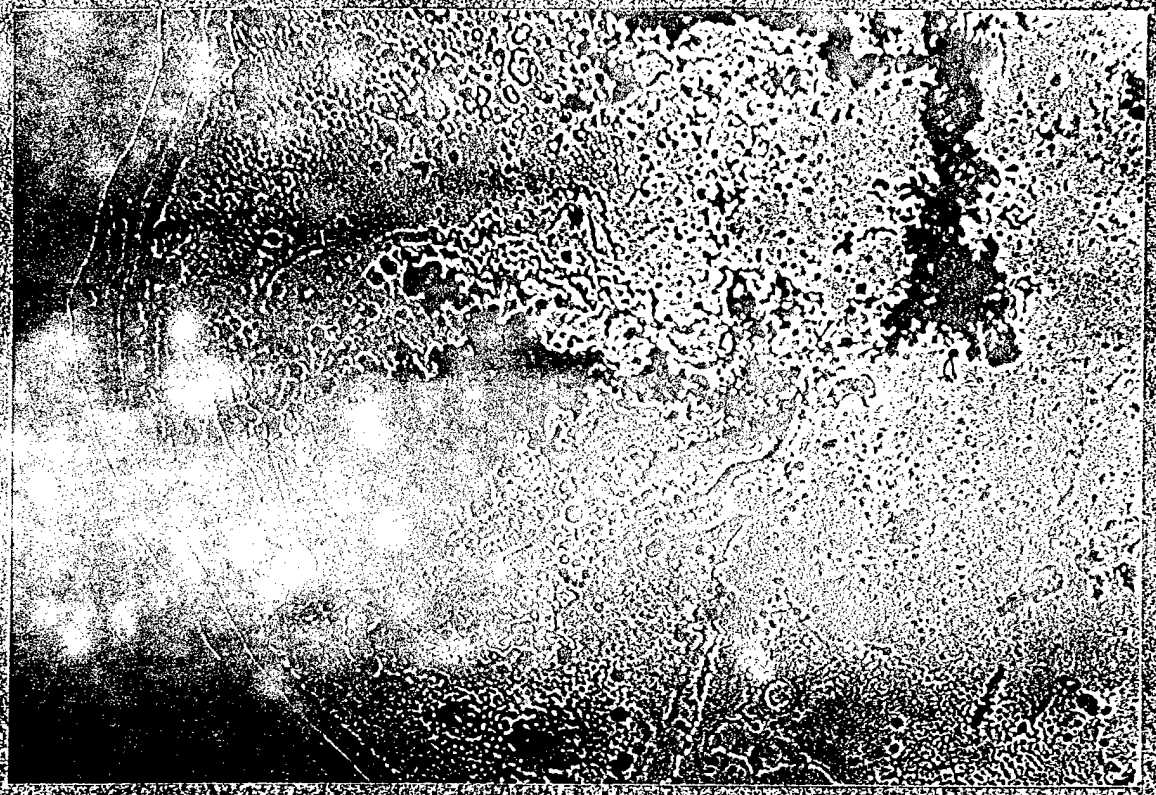


Photomicrograph 68



Photomicrograph 69

Photomicrograph 70



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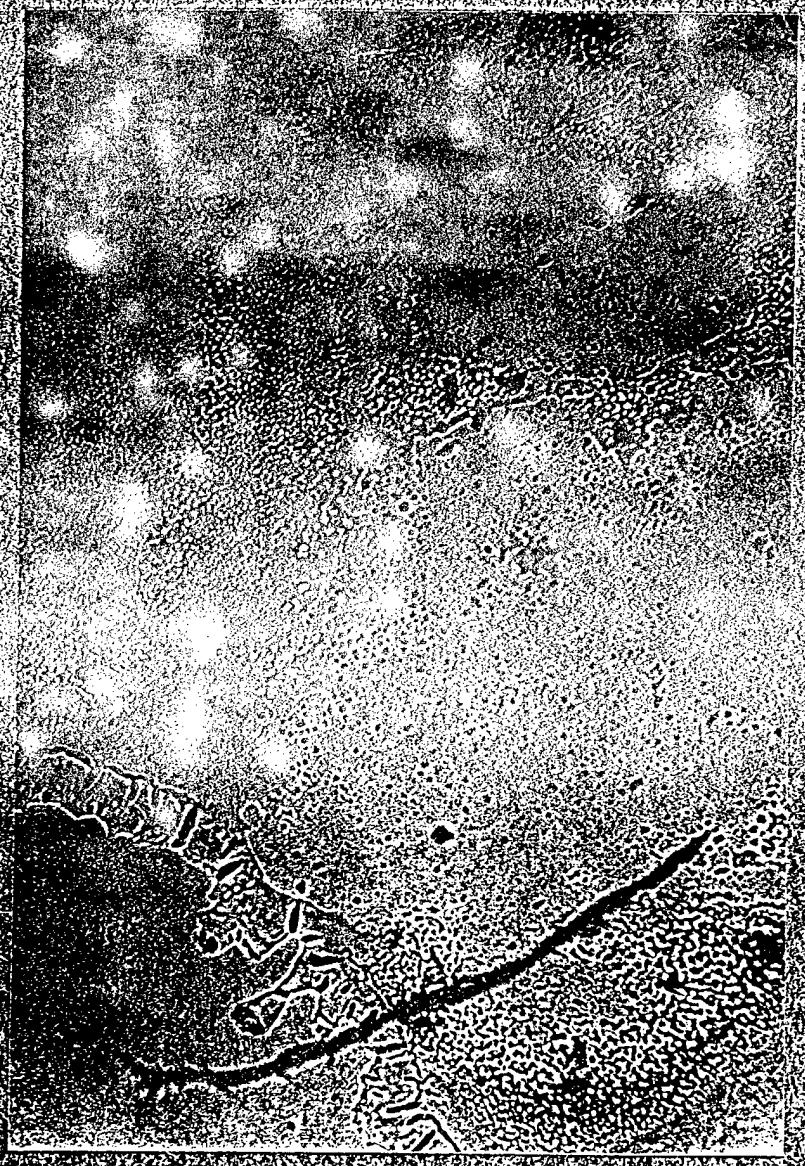

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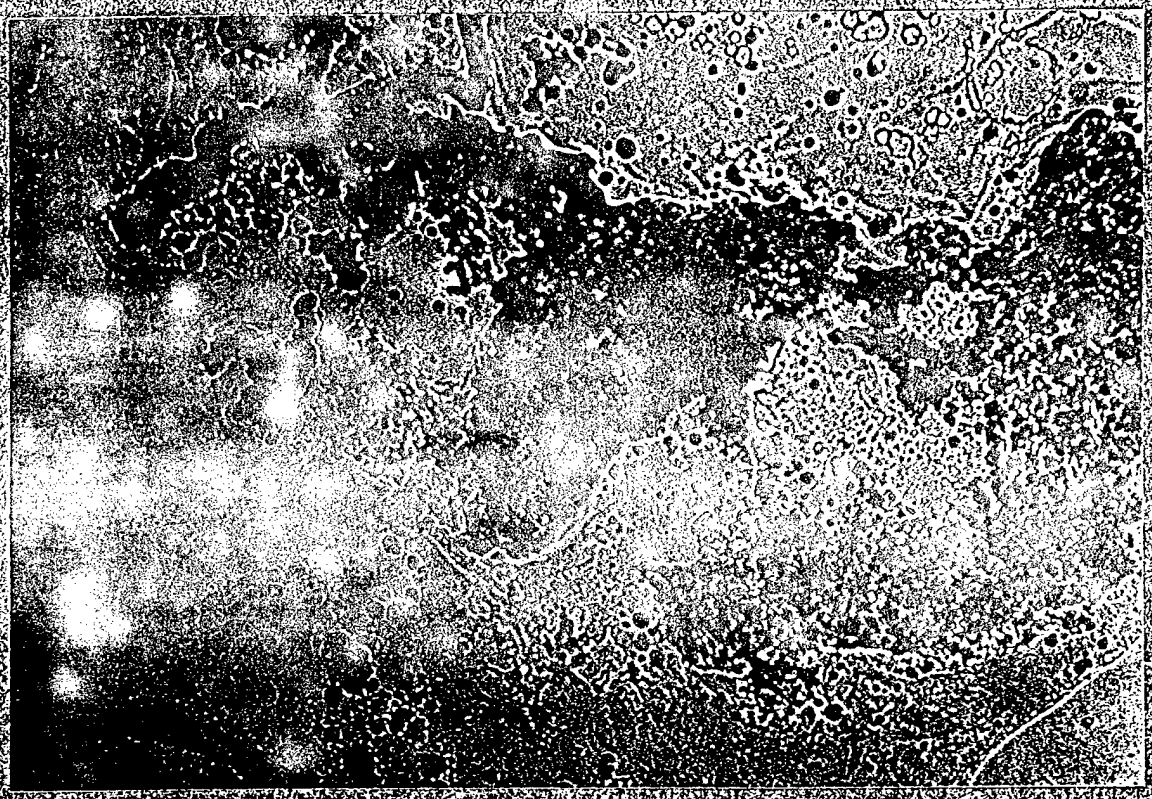
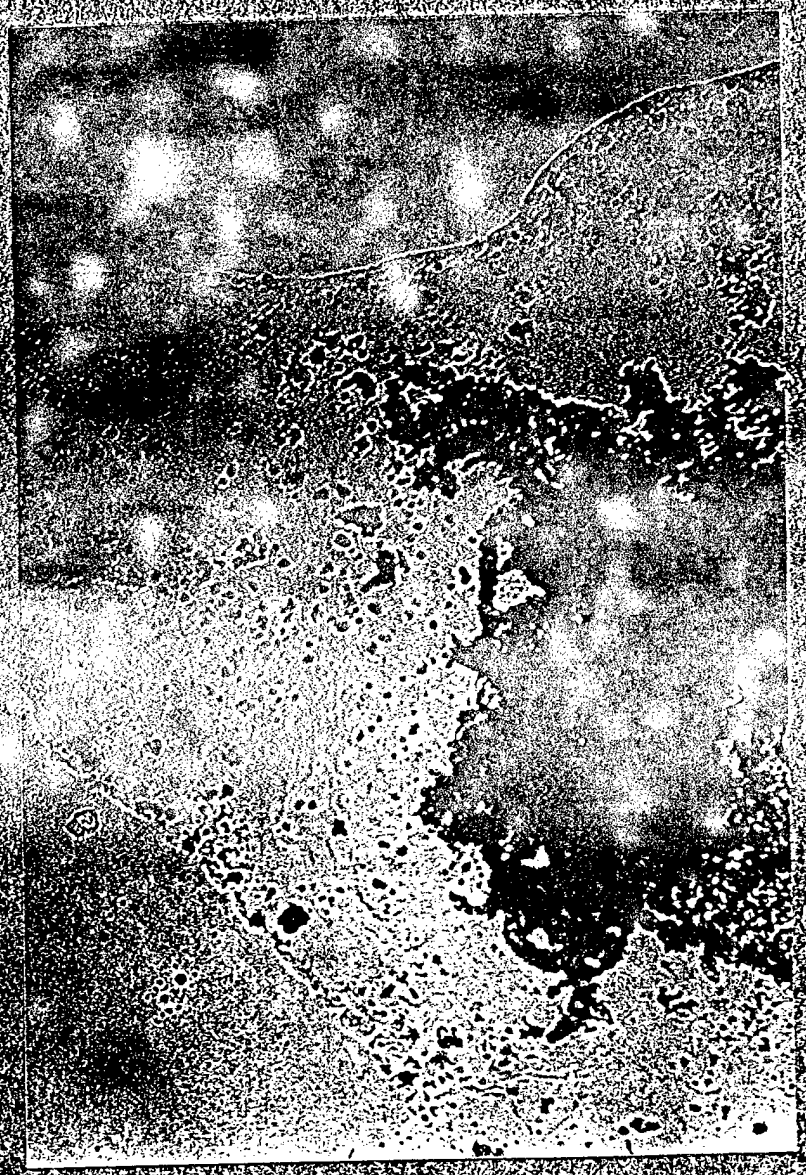


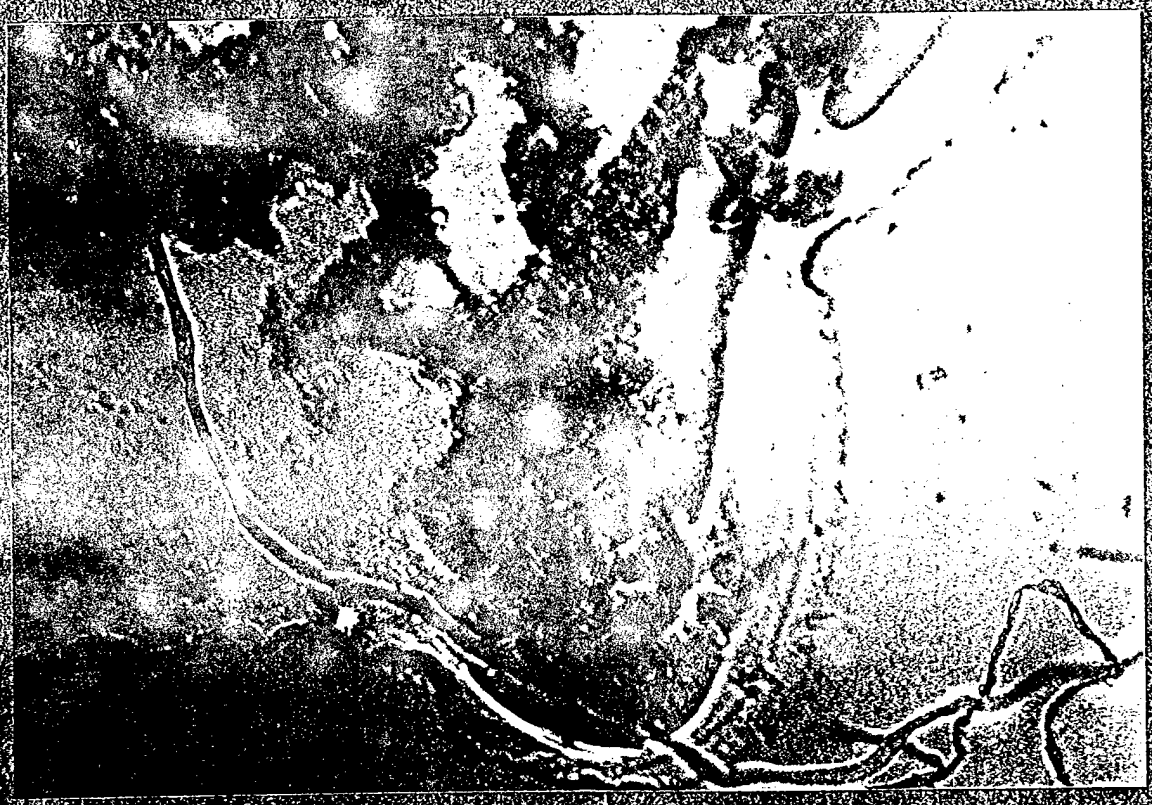
Photomicrograph 73



Photomicrograph 74

Photomicrograph 75

Photomicrograph 76



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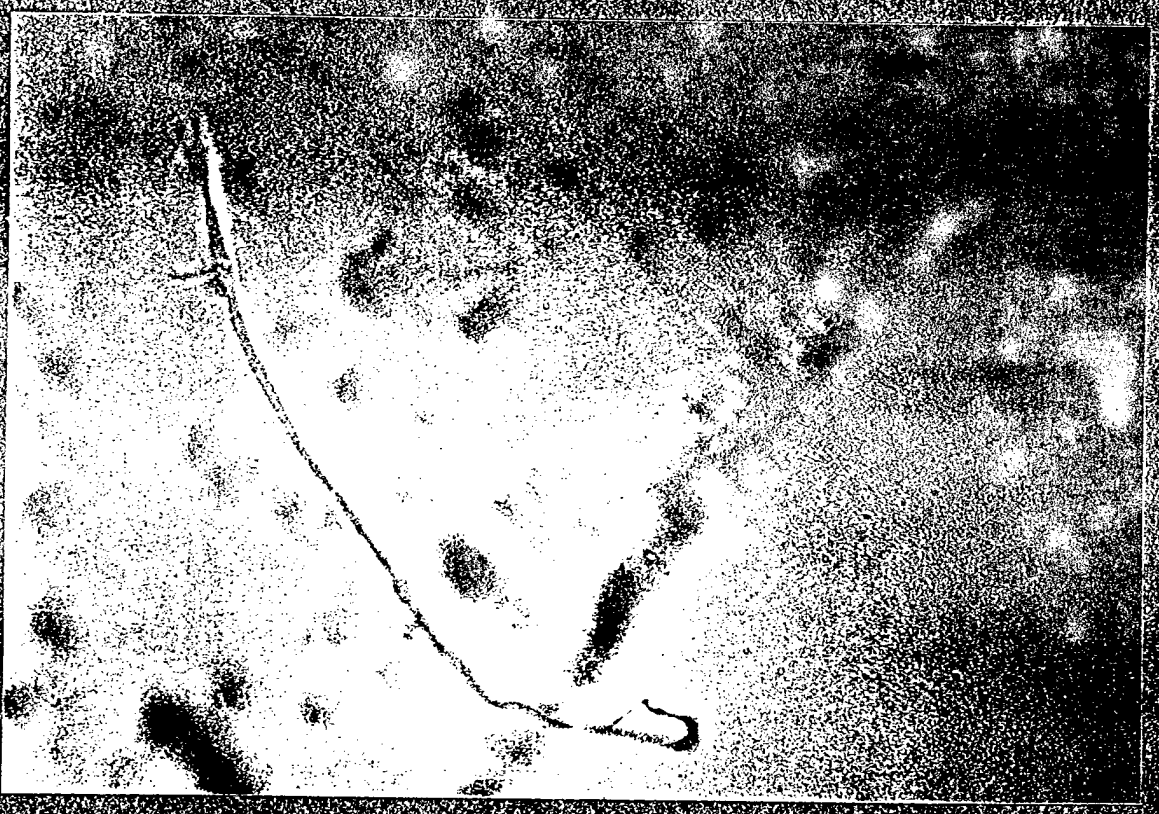

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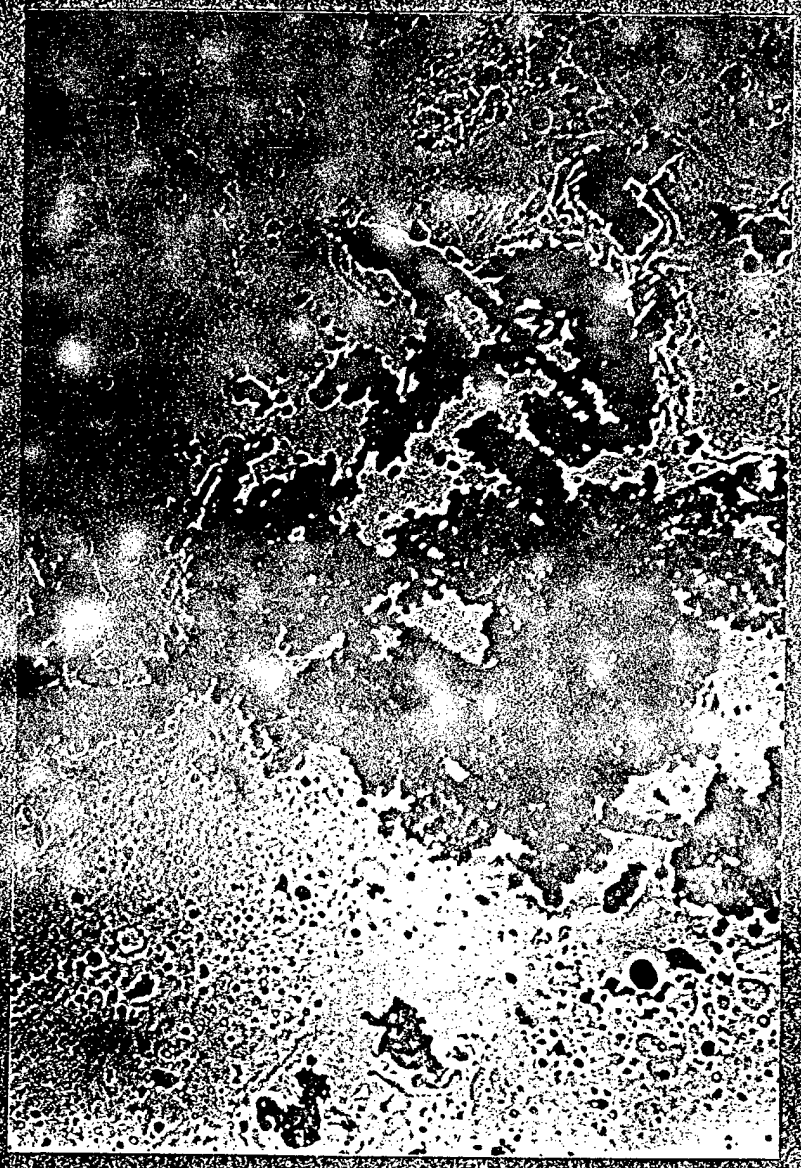

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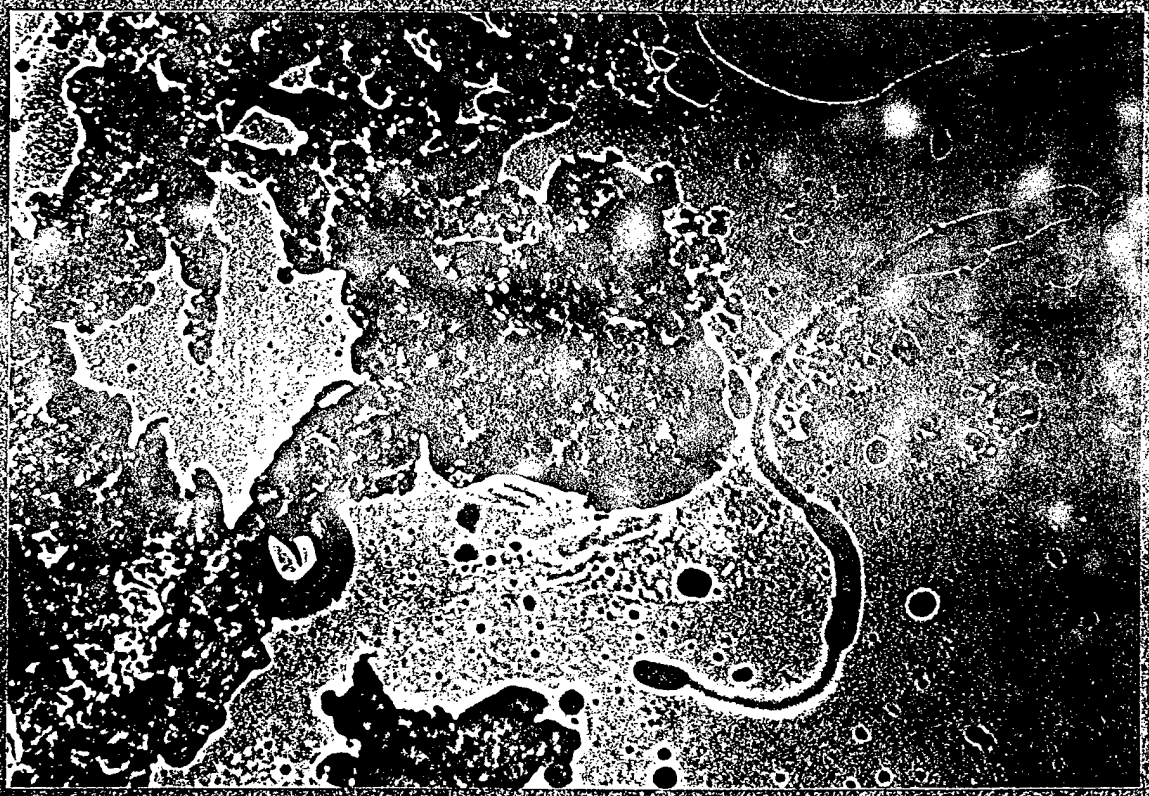
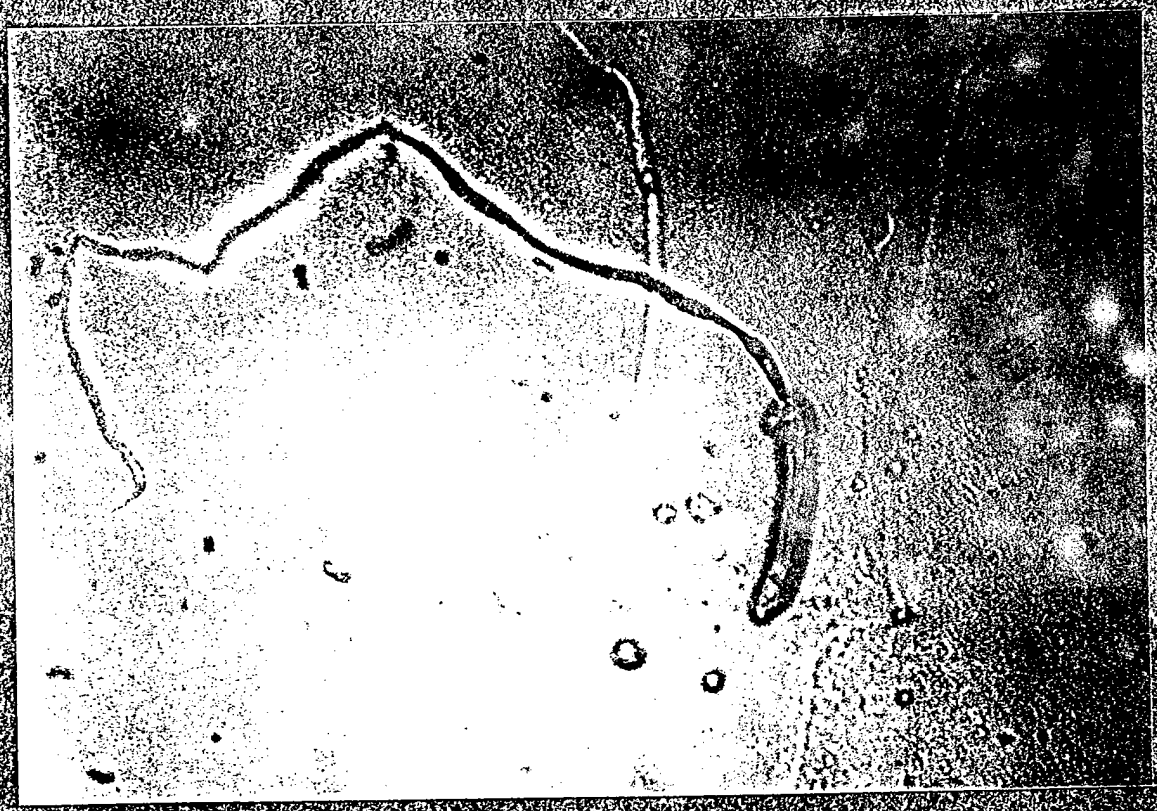


Photomicrograph 80



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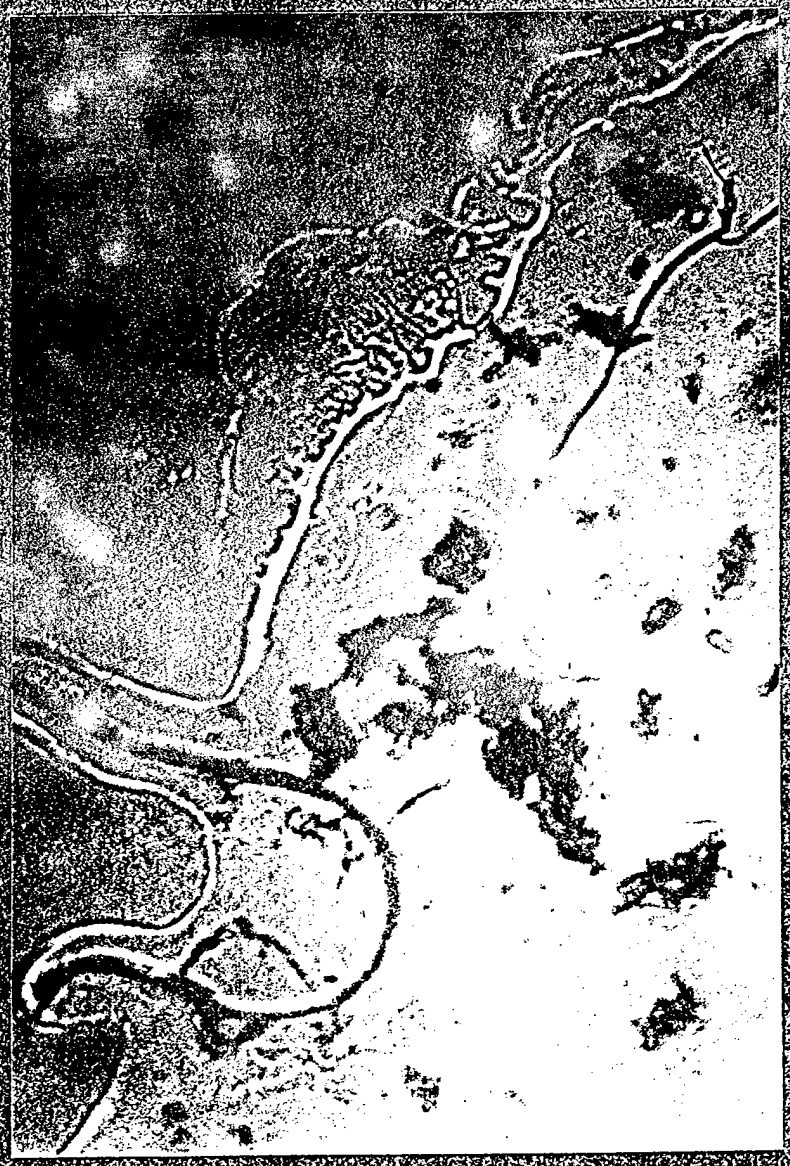

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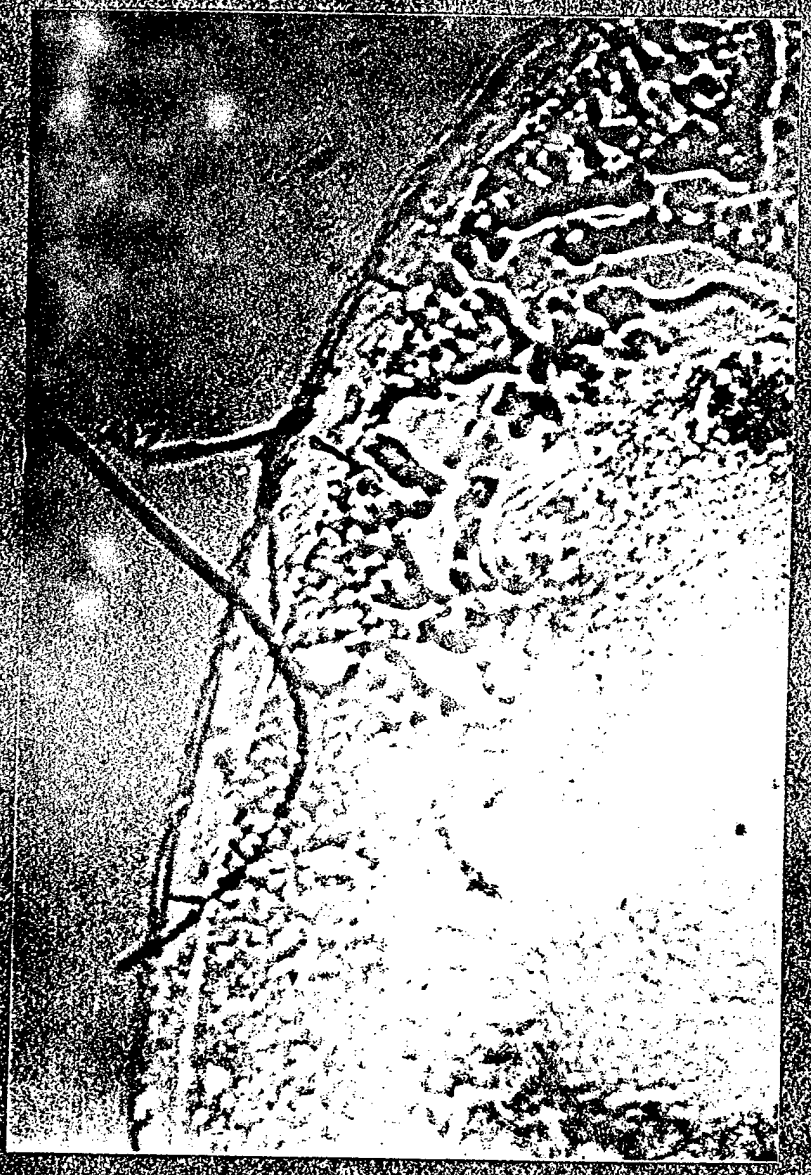

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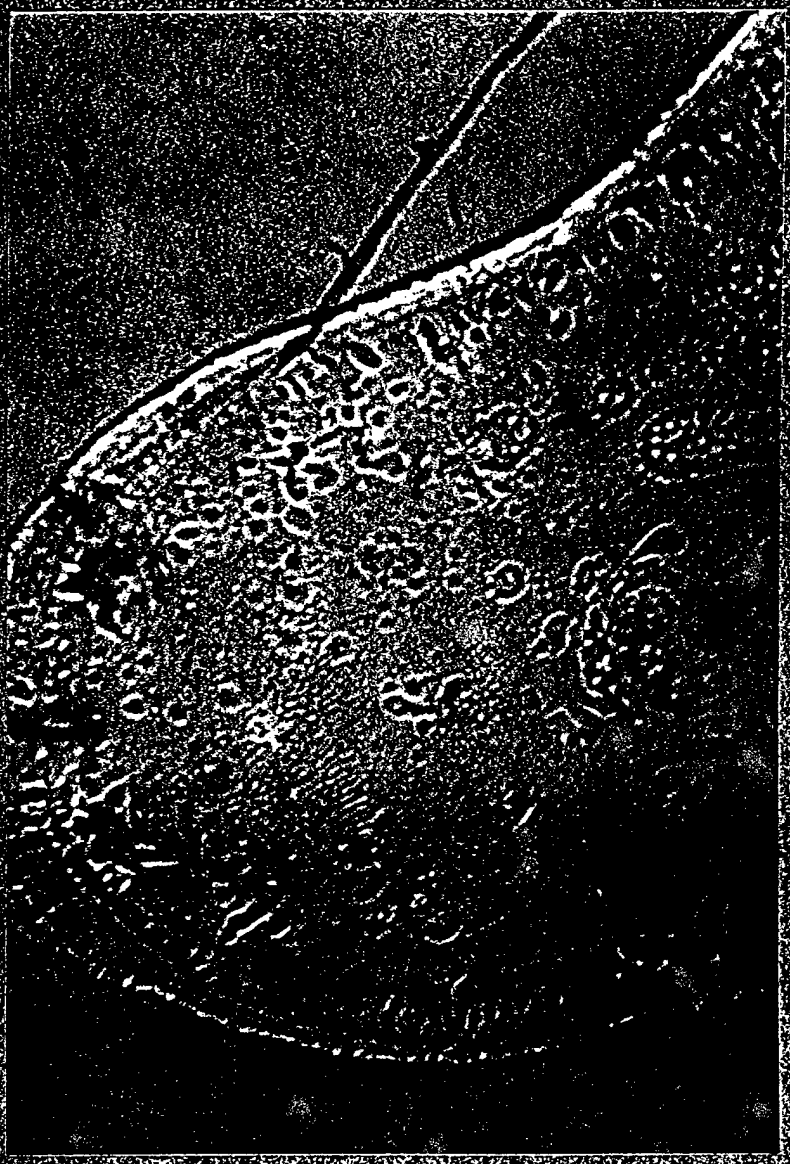


Photomicrograph 84

Photomicrograph 85

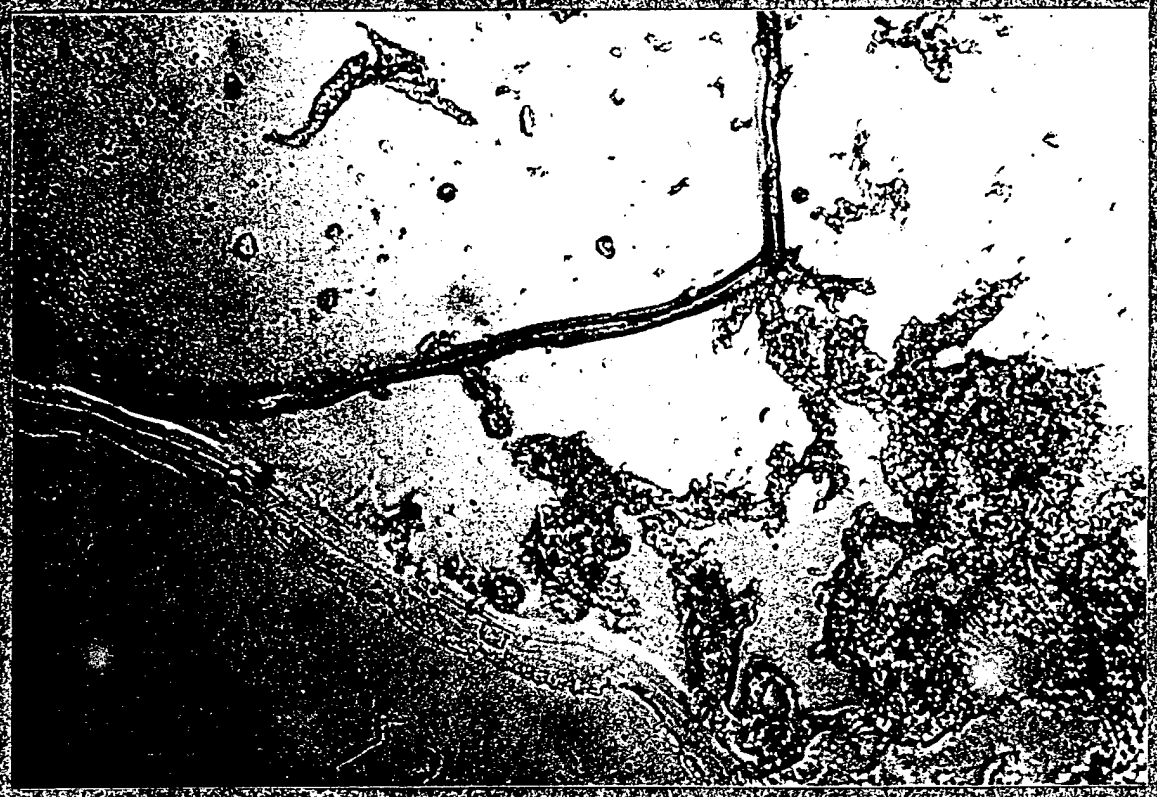
Photomicrograph 86

Photomicrograph 87

Photomicrograph 88

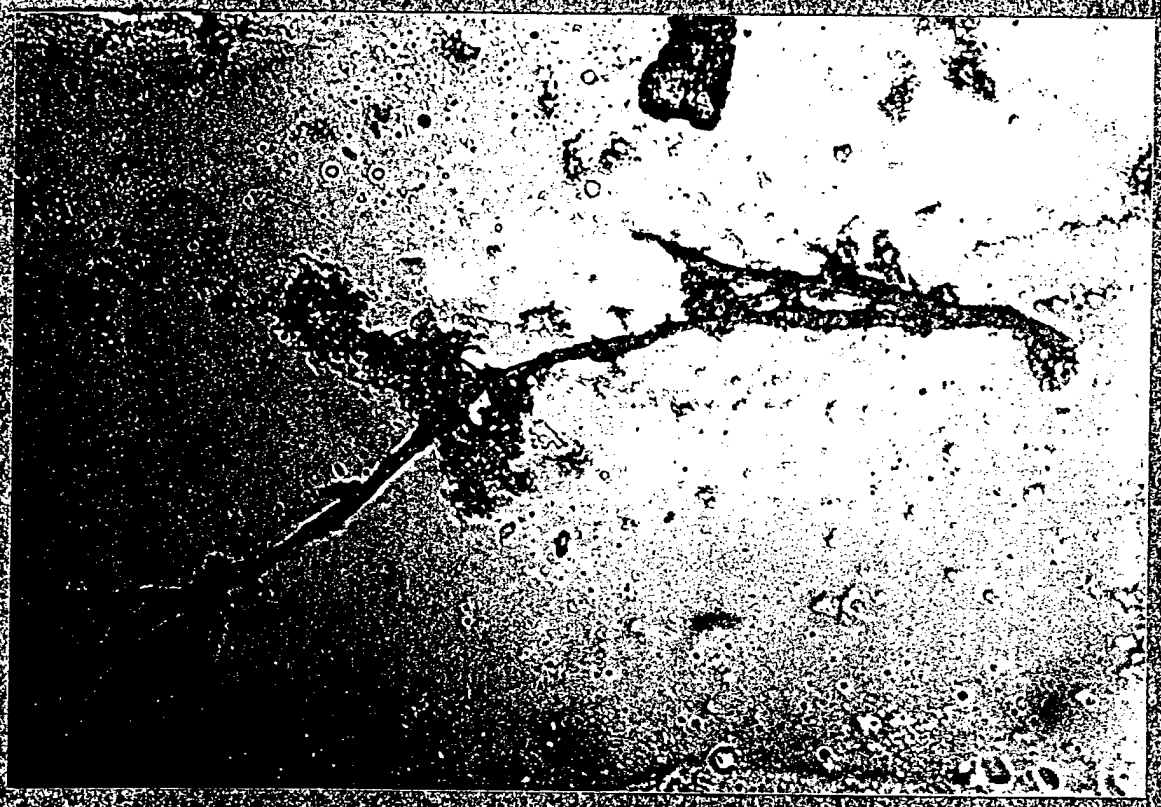
Photomicrograph 89

Page 171

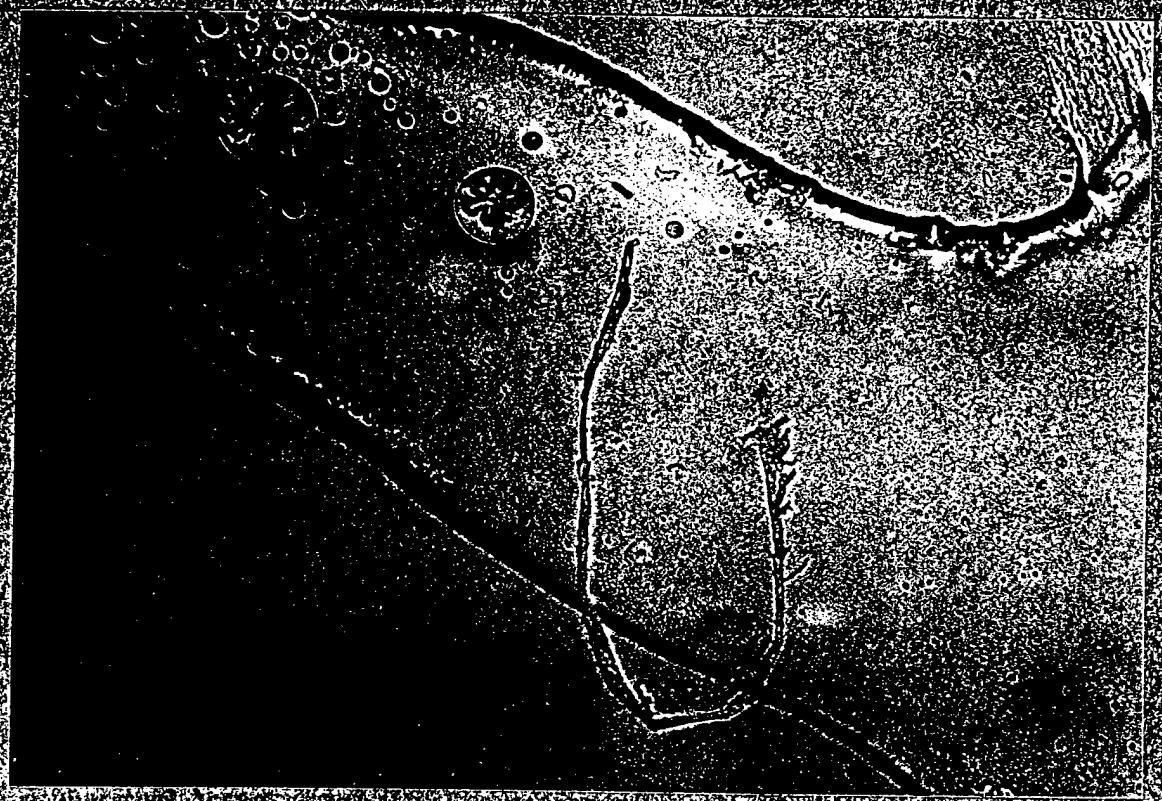


Photomicrograph 90

Page 172



Photomicrograph 91



Photomicrograph 92

CONCLUSIONS

I conclude that the hypothesis was supported by the results of this study. That is, it is possible to detect sub-lethal concentrations of neurotoxicants by image analysis of neural tissue using voltage-sensitive fluorescent dyes and by anterograde/retrograde tracing of injected tracers. This surprising results suggests an expansion of the scope of the work to include further testing of additional neurotoxicants and validation of these results and future results by an optics setup with a smaller working distance (ie. confocal microscope). Testing of the original subjects of the study, excitatory amino acids will probably require microinjection of the compound into the embryo/tadpole.

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MEETINGS ATTENDED (NONE) No Travel money

MSK #178 EFFECTS OF α-CHYMOTRYPSIN AND FOLIC ACID ON THE MEMBRANE POTENTIAL OF ALBINO EMBRYOS BURNS 3-31-95

GROUP 1 = BLEEDINGS OF #20 ♀ & #11 ♂ GROUP 2 = BLEEDINGS OF #21 ♀ & #12 ♂

SCAN No	SAMPLE DESCRIPTION	INT. 2A	% OF NEG. CONTROL	% OF MAX. RESPONSE
1	NEGATIVE CONTROL : GROUP 1	7.9380×10^4	$\bar{x} = 7.252 \times 10^4$	22.67
2	POSITIVE CONTROL : 50 mg/L α-CHYMOTRYPSIN	35.021×10^4	482.92	100.00*
3	POSITIVE CONTROL : 40 mg/L FOLIC ACID	13.093×10^4	180.54	37.39
4	POSITIVE CONTROL : 10 mg/L α-CHYMOTRYPSIN	8.6494×10^4	119.27	24.70
5	POSITIVE CONTROL : 5 mg/L α-CHYMOTRYPSIN	11.2300×10^4	154.85	32.07
6	POSITIVE CONTROL : 2.5 mg/L α-CHYMOTRYPSIN	8.1901×10^4	112.94	23.39
7	NEGATIVE CONTROL : GROUP 2	3.9899×10^4	———	
8	POSITIVE CONTROL : 50 mg/L α-CHYMOTRYPSIN	3.0162×10^5	755.96	
9	POSITIVE CONTROL : 40 mg/L FOLIC ACID	4.4055×10^4	110.42	
10	POSITIVE CONTROL : 10 mg/L α-CHYMOTRYPSIN	5.0714×10^4	127.11	
11	POSITIVE CONTROL : 5 mg/L α-CHYMOTRYPSIN	5.7288×10^4	143.58	
12	POSITIVE CONTROL : 2.5 mg/L α-CHYMOTRYPSIN	4.8205×10^4	120.82	
13	GROUP 1 : 50 mg/L α-C AND 2.5 mg/L FA	1.1298×10^5	155.79	3.28
14	" " "	1.8445×10^5	254.34	5.27
15	NO SCAN	———	———	———
16	GROUP 1 : 50 mg/L α-C AND 5 mg/L FA	1.874×10^5	258.41	5.35
17	" " "	1.5673×10^5	216.12	4.48
18	NO SCAN	———	———	———
19	GROUP 1 : 50 mg/L α-C AND 10 mg/L FA	2.7333×10^5	376.90	7.80
20	" " "	1.3397×10^5	184.74	3.83
21	NO SCAN	———	———	———
22	GROUP 1 : 50 mg/L α-C AND 20 mg/L FA	1.5133×10^5	208.67	4.32
23	" " "	9.749×10^4	134.43	27.84
24	NO SCAN	———	———	———
25	GROUP 1 : 50 mg/L α-C AND 40 mg/L FA	1.1350×10^5	156.52	3.24
26	" " "	1.2823×10^5	176.82	3.66
27	NO SCAN	———	———	———
28	NEGATIVE CONTROL : GROUP 1	6.4538×10^4	———	18.43
29	NEGATIVE CONTROL : GROUP 1	7.3632×10^4	———	21.03

NOTE : THE PERCENT OF MAXIMUM RESPONSE WAS CALCULATED INCORRECTLY. SEE p. 75.

5-3-95

Naphthalene on African embryos
DISK #180 – 30min. incubation, di-4 ANNEPS

SCAN	CONCENTRATION	~~Intercept SLOPE~~	% CONTROL	
1	Neg. control	4.52×10^3	*	
2	50mg/L α-Chaconine	1.98×10^4	~~43.8%~~	438%
3	1.25 mg/L Naphthalene	3.99×10^3	88%	
4	2.50 mg/L Naphthalene	6.13×10^3	~~13.6%~~	136%
5	5 mg/L "	6.52×10^3	~~14.4%~~	144%
6	10mg/L "	3.84×10^3	85%	
7	15 mg/L "	7.73×10^3	~~17.1%~~	171%
8	30 mg/L "	9.01×10^3	~~19.9%~~	199%
9	Neg: control	8.33×10^3		
10	50mg/L α-Chaconine	1.41×10^4	~~13.2%~~ 31	312%

Made a graph + saved as Naph5395.IPG on DISK #194

5/9/95
Discarded all above data because exit monochromator was set at 525. Kept all data above on disk though.

Naphthalene on Albino embryos
DISK #180 – 30 min. incubation in di-4

SCAN	CONCENTRATION	Intercept
1A	Neg. Control	– No GOOD - Changed monochromator in time for 3rd Scran
2A	50mg/L α-Chaconine	– No GOOD - Changed monochromator
3A	1.25 mg/L Naphthalene	9.64×10^4
4A	2.50 mg/L Naph.	8.52×10^4
5A	5.0 mg/L Naph.	9.94×10^4
6A	10mg/L Naph.	9.02×10^4
7A	15mg/L Naph.	8.22×10^4
8A	30mg/L Naph	1.01×10^5
9A	Neg. Control	1.05×10^5 } 1.06×10^5
10A	Neg. Control	1.07×10^5 }
11A	50mg/L α-Chaconine	4.26×10^5

Made graph + saved as NAPH5995.IPG on DISK #194

5-12-95 Friday

FETAX experiment with Naphthalene (30mg/L) on African embryos.

DISH #	CONCENTRATION	#survived 24h.	48h.	72h.	96h.
1	Neg. Control			24 (5 deformed)	
2	Neg. Control			21 (1 deformed)	
3	50mg/L α-Chac.			~~0~~	
4	50mg/L α-Chac.			~~0~~	
5	1.25mg/L Naphthalene			~~0~~	
6	1.25mg/L "			23 (2 deformed)	
7	2.5mg/L "			21	
8	2.5mg/L "			21	
9	5.0mg/L "			24	
10	5.0mg/L "			18 (4 deformed)	
11	10.0mg/L "			24	
12	10.0mg/L "			21 (1 deformed)	
13	15.0mg/L "			21	
14	15.0mg/L "			21 (2 deformed)	
15	30.0mg/L "			25 (3 deformed)	
16	30.0mg/L "			23	

Made spreadsheet + saved as NAPH5.12.95 on DISK 194

Embryos (25) were put into dishes at 1:30pm Friday.

5/~~26~~27/95 ~~Friday~~

FETAX experiment with Kainic acid (50mg/L) on Albino embryos.

DISH #	CONCEN.	24h.	48h	72h	96h
1	50mg/L Kainic acid	14 surv.			11
2	25mg/L Kainic acid	12 surv.			12

At 24h. - embryos were still growing, switched water (put new water in each dish.)

5/26/95 Friday

Kainic Acid on Albino embryos (50mg/L KA stock)
Disk #182 - 30 min. incub., di-4 ANEPPS

SCAN	CONCEN.	Intercept	
1	NC	4.75×10^4	4.86×10^4
2	NC	4.63×10^4	
3	NC	5.20×10^4	
4	5mg/L KA	4.58×10^4	
5	15mg/L KA	6.11×10^4	
6	25mg/L KA	5.99×10^4	
7	50mg/L KA	5.78×10^4	
8	50mg/L α-Chac.	2.79×10^5	
9	2.5mg/L KA	5.51×10^4	

Graphed + saved as KA-52695. ~~IAG~~ on Disk #194

5/31/95

Kainic Acid on normal embryos (From Mendi) - 50mg/L stock
(FETAX experiment)

DISH#	CONCENTRATION	0	# SURVIVED 24h.	48h	72h	96h
1	NC (10ml FTX)	20	20	19	19	19
2	NC (10ml FTX)	20	19	19	18	18
3	NC (10ml FTX)	20	19	18	18	18
4	0.2ml KA (9.8ml FX)	20	20	20	20	20
5	0.2ml KA "	20	19	19	19	19
6	0.2ml KA "	20	19	19	19	19
7	1ml KA (9ml FTX)	20	+9 18	18	18	18
8	1ml KA "	20	20	20	20	20
9	1ml KA "	20	20	20	20	20
10	2ml KA (8ml FTX)	20	20	19	19	19
11	2ml KA "	20	20	19	19	19
12	2ml KA "	20	20	20	20	20
13	5ml KA (5ml FTX)	20	20	20	20	20
14	5ml KA "	20	17	17	16	16
15	5ml KA "	20	19	18	17	17
16	10ml KA (0ml FTX)	20	19	19	19	18
17	10ml KA "	20	20	20	20	20
18	10ml KA "	20	20	20	20	20
19	1ml α-Chac. (9ml FTX)	20	16	0	0	0
20	1ml α-Chac. "	20	12	0	0	0

put in 30% formalin on last day

These embryos (previous page) had already started neuralating.
Made into spreedsheet & saved as KA_531 ~~53195~~.WQ1 on disk 194

FETAX experiment with normal embryos (from Mendi) using Kainic Acid. Done earlier on 5/31/95.

DISH#	CONCENTRATION	# SURVIVED 0	24h	48h	72h	96h
1	NC	25	25	24	24	24
2	NC	25	25	25	23	23
3	50mg/L KA	25	25	25	25	25
4	50mg/L KA	25	25	25	25	~~25~~ 24
5	25mg/L KA	25	25	25	25	25
6	25mg/L KA	25	24	23	23	23
7	12.5mg/L KA	25	24	24	24	24
8	12.5mg/L KA	25	23	23	23	23

Made into spreedsheet & saved as KA53195.WQ1 on disk 194

6/1/95
FETAX experiment with Colchicine (50mg/L stock) using African embryos. Started first part of test at 3:20pm and second part at 4:30pm.

DISH#	CONCEN.	0	24h.	48h.	72h	96h	
1	NC	25	25	25	25	25	Put in 3% Formalin on last day
2	NC	25	24	23	23	23	
3	0.2ml Colchicine	25	25	25	25	25	
4	0.2ml "	25	25	24	24	24	
5	1ml "	25	25	22	22	22	
6	1ml "	25	25	25	25	25	
7	2ml "	25	25	24	24	24	
8	2ml "	25	25	25	25	25	
9	5ml "	25	25	23	23	23	Saved as spreadsheet as COL_6-1.WQ1 on disk 194
10	5ml "	25	25	24	24	24	
11	10ml "	25	25	24	24	23	
12	10ml "	25	25	25	25	25	
13	50mg/L → 1ml & Chac.	25	18	6	0	0	

6/1/95 continued

SECOND PART

DISH#	CONCEN.	0	24h	48h	72h	96h	
14	NC	25	25	25	25	25	
15	NC	25	25	25	25	25	Put in 3% Formalin on last day
16	0.2ml Colchicine	25	25	24	22	21	
17	0.2ml "	25	25	25	25	25	
18	1ml "	25	24	24	24	24	
19	1ml "	25	25	23	23	23	
20	2ml "	25	25	~~24~~25	24	24	
21	2ml "	25	25	25	25	25	Made into spreadsheet + saved as COL_6-1.WK1 on 6/6/95
22	5ml "	25	25	25	25	25	
23	5ml "	25	25	25	~~25~~24	23	
24	10ml "	25	25	25	25	25	
25	10ml "	25	25	25	25	25	
26	(50mg/L) 1ml d-Chac	25	24	9	0	0	

FETAX experiment with Kainic Acid + Colchicine using African embryos. Started this test at 4:10pm. Diluted the stocks from 50mg/L to 5mg/L by taking 25mls of 50mg/L stock (KA + Colchicine), putting in 250ml volumetric flask + bring up to line with Fetax solution.

DISH#	CONCEN.	0	24h	48h	72h	96h	
1	NC	25	24	24	24	24	
2	NC	25	25	25	25	24	
3	5mg/L KA (0ml FTX)	25	25	25	25	25	put in 3% formalin on last day
4	5mg/L KA "	25	25	25	25	25	
5	2.5mg/L KA (5ml FTX)	25	25	25	25	25	
6	2.5mg/L KA "	25	25	24	24	24	
7	1mg/L KA (8ml FTX)	25	24	24	24	~~24~~23	
8	1mg/L KA "	25	25	24	24	24	
9	0.5mg/L KA (9ml FTX)	25	25	23	23	22	
10	0.5mg/L KA "	25	25	25	25	25	Made into spreadsheet + saved as KA-6-1.WK1 on 6/6/95
11	0.25mg/L KA (9.5ml FTX)	25	25	25	24	24	
12	0.25mg/L KA (")	25	26	24	23	23	
13	0.125mg/L KA (9.75ml FTX)	25	24	23	23	22	
14	0.125mg/L KA "	25	~~24~~25	24	24	24	

6/1/95 continued

DISH#	CONCEN.	0	24h	48h	72h	96h	
15	(9.9ml FTX) 0.05mg/L KA	25	24	24	24	24	
16	" 0.05mg/L KA	25	25	23	23	23	
17	NC (10ml FTX)	25	24	23	23	23	
18	NC "	25	25	25	25	25	
19	5mg/L Colchicine	25	25	25	25	25	put in
20	5mg/L "	25	25	25	24	24	3%
21	2.5mg/L "	25	25	25	25	25	Formal
22	2.5mg/L "	25	25	25	24	24	on las
23	1mg/L "	25	25	24	24	24	day
24	1mg/L "	25	25	25	25	25	
25	0.5mg/L "	25	25	24	24	24	
26	0.5mg/L "	25	25	25	25	25	Made into spreadsheet
27	0.25mg/L "	25	25	25	24	24	+ saved
28	0.25mg/L	~~25~~24 25	~~24~~25	25	25	25	Colchicine
29	0.125mg/L "	25	25	25	25	25	on DBK1
30	0.125mg/L "	25	25	25	25	25	
31	0.05mg/L "	25	25	24	24	23	
32	0.05mg/L "	25	25	25	25	25	

6/8/95

Colchicine (new supply) on albino embryos (50mg/L)
DISK #183 - 30 min. incub. in di-4 ANEPPS

SCAN	CONCENTRATION	Intercepts
1,7,13,	NC	7.81×10^4, 9.6×10^4, 8.57×10^4 = 78.66
2,8,14,	5mg/L Colchicine	9.53×10^4, 6.73×10^4, 8.27×10^4 = 78.18
3,9,15,	15mg/L Colchicine	8.57×10^4, 6.57×10^4, 6.95×10^4 = 77.36
4,10,16,	25mg/L "	7.97×10^4, 7.51×10^4, 6.99×10^4 = 77.49
5,11,17,	50mg/L "	5.15×10^4, 7.07×10^4, 7.16×10^4 = 77.46
6,12,18	50mg/L α-Chac.	3.75×10^5, 3.97×10^5, 3.09×10^5 = 73.60

graphed + saved as COL_6895.IPG on DBK 194

FETAX experiment with Colchizine (new - 50mg/L stock) on albino embryos

DISH#	CONCEN.	(#) SURVIVAL 0	24	48	72	96
1	NC	25	25	19	18	18
2	5 mg/L Colchizine	25	25	22	21	21
3	15 mg/L "	25	25	18	17	17
4	25 mg/L "	25	25	21	19	19
5	50 mg/L "	25	25	22	21	19
6	50 mg/L α-Chaconine	25	0	0	0	0

* Put in 3% Formalin on last day

Made into spreadsheet + Saved as COL_6-8.WQ1 on DISK 194

7/12/95

Glycine and Glutamate on albino embryos (~8-9 hours old) - 50mg/L stock For each, 30 min. incubation in 20 ul di-4, saved on DISK #187.

SCAN	CONC.	% Control	Intercepts	
1	NC		1.37×10^5	(ave. = 1.465)
2	PC - α-Chac. (50 mg/L) 5/95		8.38×10^5	
3-5	50mg/L glycine (pH=8)	97.2	1.35×10^5, 1.32×10^5, 1.64×10^5	=> 1.44
6-8	25mg/L "	96.6	1.42×10^5, 1.28×10^5, 1.64×10^5	=> 1.45
9-11	10mg/L "	91.5	1.56×10^5, 1.43×10^5, 1.60×10^5	=> 1.53
12-14	5 mg/L "	85.4	2.07×10^5, 1.49×10^5, 1.37×10^5	=> 1.64
15-17	2.5 mg/L "	88.1	1.65×10^5, 1.67×10^5, 1.46×10^5	=> 1.59
18-20	1.25 mg/L "	81.4	1.62×10^5, 1.89×10^5, 1.67×10^5	=> 1.72
21-23	NC		1.27×10^5, 1.59×10^5, 1.44×10^5	=> 1.43
24,25	50mg/L glutamate (pH=8)	122.8	1.16×10^5, 1.11×10^5	=> 1.14
27,28	25 mg/L "	119.7	1.15×10^5, 1.19×10^5	=> 1.17
30,31	10 mg/L "	116.7	1.16×10^5, 1.23×10^5	=> 1.20
33,34	5 mg/L "	102.2	1.24×10^5, 1.50×10^5	=> 1.37
36,37	2.5 mg/L "	117.6	1.23×10^5, 1.15×10^5	=> 1.19
39,40	1.25 mg/L "	107.7	1.18×10^5, 1.41×10^5	=> 1.30
43-45	NC		1.18×10^5, 1.24×10^5	=> 1.21
46,47	25mg/L mimosine (pH=7)		1.62×10^5, 1.40×10^5	=> 1.51

Graphed + Saved as Glycine.IPG + Glutamat.IPG on DISK 194

7/14/95

Homocysteine on albino embryos (~8-9 hours old) - 50 mg/L stock, 30 min. inc. in 20 ml di-4, saved on DISK #188.

SCAN	Conc.	Intercepts	graphed + saved as HOMOCYST.IPG on DISK 194
1-3	NC	1.11×10^5, 1.49×10^5, 1.31×10^5	$\Rightarrow 1.30_{10^5}$
4-6	50 mg/L homocysteine (pH=8)	1.40×10^5, 1.20×10^5, 1.22×10^5	$\Rightarrow 1.27_{10^5}$
7-9	25 mg/L "	1.14×10^5, 1.35×10^5, 1.22×10^5	$\Rightarrow 1.24_{10^5}$

8/7/95 TEST #1 (check page 18 For data)
FETAX experiment with α-chaconine and Folic acid on normal/African embryos from Mendi + Jason. Embryos were starting to neuralate while setting up test. Folic acid stock was 100mg/L and α-Chac. stock was 50mg/L. Folic Acid, α-Chac. + Fetax solution was at ~6 pH. Started test around 4:00pm + covered dishes with foil after test was setup. (** note: embryos were mixed together From 3 pairs of Frogs)

DISH#	Concentration	ml α-C	ml FA	ml FTX	Survival 0	24	48	72	96
1	NC	0	0	10	25	25	25	24	24
2	NC	0	0	10	25	25	25	24	24
3	NC	0	0	10	25	25	25	25	25
4	1mg/L FA, ~~2.5~~ mg/L α-C	0	0.1	9.9	25	24	21	20	20
5	"	0	0.1	9.9	25	24	23	23	23
6	"	0	0.1	9.9	25	25	24	23	22
7	2.5mg/L FA, ~~2.5~~ mg/L α-C	0	0.25	9.75	25	25	25	24	24
8	"	0	0.25	9.75	25	25	25	25	24
9	"	0	0.25	9.75	25	25	24	24	24
10	5mg/L FA, ~~2.5~~ mg/L α-C	0	0.5	9.5	25	25	25	24	24
11	"	0	0.5	9.5	25	25	23	22	22
12	"	0	0.5	9.5	25	25	25	25	25
13	10mg/L FA, ~~2.5~~ mg/L α-C	0	1	9	25	25	24	23	23
14	"	0	1	9	25	25	24	23	23
15	"	0	1	9	25	25	22	21	21
16	20mg/L FA, ~~2.5~~ mg/L α-C	0	2	8	25	25	24	24	24
17	"	0	2	8	25	25	23	23	23
18	"	0	2	8	25	25	24	22	22

Vial #	Concentration	ml d-C	ml FA	ml FTX	0	Survival 24	48	72	96
19	50mg/L FA, ~~25~~ 0 mg/L d-C	0	5	5	25	25	24	23	23
20	"	0	5	5	25	25	23	23	23
21	"	0	5	5	25	24	22	22	22
22	0mg/L FA, 2.5mg/L d-C	0.5	0	9.5	25	25	17	16	15
23	"	0.5	0	9.5	25	25	20	20	14
24	"	0.5	0	9.5	25	24	10	10	5
25	1mg/L FA, 2.5mg/L d-C	0.5	0.1	9.4	25	25	20	19	18
26	"	0.5	0.1	9.4	25	25	15	13	10
27	"	0.5	0.1	9.4	25	25	17	15	15
28	2.5mg/L FA, 2.5mg/L d-C	0.5	0.25	9.25	25	25	20	17	15
29	"	0.5	0.25	9.25	25	23	19	18	16
30	"	0.5	0.25	9.25	25	25	14	12	7
31	5mg/L FA, 2.5mg/L d-C	0.5	0.5	9	25	25	18	18	15
32	"	0.5	0.5	9	25	25	15	15	14
33	"	0.5	0.5	9	25	25	21	21	21
34	10mg/L FA, 2.5mg/L d-C	0.5	1	8.5	25	24	16	14	13
35	"	0.5	1	8.5	25	25	12	12	10
36	"	0.5	1	8.5	25	25	17	15	14
37	20mg/L FA, 2.5mg/L d-C	0.5	2	7.5	25	25	18	15	14
38	"	0.5	2	7.5	25	25	14	14	14
39	"	0.5	2	7.5	25	25	16	14	11
40	50mg/L FA, 2.5mg/L d-C	0.5	5	4.5	25	24	20	19	18
41	"	0.5	5	4.5	25	25	20	18	17
42	"	0.5	5	4.5	25	25	16	13	12
43	0mg/L FA, 5mg/L d-C	1	0	9	25	23 ~~25~~	1	0	0
44	"	1	0	9	25	25	~~5~~ 6	6	0
45	"	1	0	9	25	25	1	0	0
46	1mg/L FA, 5mg/L d-C	1	0.1	8.9	25	24	1	1	0
47	"	1	0.1	8.9	25	25	11	8	4
48	"	1	0.1	8.9	25	25	0	0	0
49	2.5mg/L FA, 5mg/L d-C	1	0.25	8.75	25	24	0	0	0
50	"	1	0.25	8.75	25	24	0	0	0
51	"	1	0.25	8.75	25	25	1	0	0
52	5mg/L FA, 5mg/L d-C	1	0.5	8.5	25	25	0	0	0
53	"	1	0.5	8.5	25	25	0	0	0
54	"	1	0.5	8.5	25	23	1	1	0
55	10mg/L FA, 5mg/L d-C	1	1	8	25	23	1	0	0
56	"	1	1	8	25	24	1	0	0
57		1	1	8	25	25	0	0	0

Dish#	Concentration	ml α-C	ml FA	ml FW	0	24	48	72	96
58	20mg/L FA, 5mg/L α-C	1	2	7	25	24	7	2	1
59	"	1	2	7	25	24	1	1	0
60	"	1	2	7	25	25	3	2	1
61	50mg/L FA, 5mg/L α-C	1	5	4	25	25	1	1	0
62	"	1	5	4	25	23	4	1	0
63	"	1	5	4	25	25	4	2	0
64	0mg/L FA, 10mg/L α-C	2	0	8	25	0	0	0	0
65	"	2	0	8	25	0	0	0	0
66	"	2	0	8	25	0	0	0	0
67	1mg/L FA, 10mg/L α-C	2	0.1	7.9	25	0	0	0	0
68	"	2	0.1	7.9	25	0	0	0	0
69	"	2	0.1	7.9	25	0	0	0	0
70	2.5mg/L FA, 10mg/L α-C	2	0.25	7.75	25	0	0	0	0
71	"	2	0.25	7.75	25	0	0	0	0
72	"	2	0.25	7.75	25	0	0	0	0
73	5mg/L FA, 10mg/L α-C	2	0.5	7.5	25	0	0	0	0
74	"	2	0.5	7.5	25	0	0	0	0
75	"	2	0.5	7.5	25	0	0	0	0
76	10mg/L FA, 10mg/L α-C	2	1	7	25	0	0	0	0
77	"	2	1	7	25	2	0	0	0
78	"	2	1	7	25	0	0	0	0
79	20mg/L FA, 10mg/L α-C	2	2	6	25	0	0	0	0
80	"	2	2	6	25	0	0	0	0
81	"	2	2	6	25	0	0	0	0
82	50mg/L FA, 10mg/L α-C	2	5	3	25	4	0	0	0
83	"	2	5	3	25	7	0	0	0
84	"	2	5	3	25	7	0	0	0

Put in 3% Formalin on last day

Made into spreadsheets & saved as CHAC_FA.WQ1, CHAC_FA2.WQ1, CHAC_FA3.WQ1, CHAC_FA4.WQ1 } DISK 194

Started scoring on 8/22/95, then will digitize whenever computer is free.

Digitized. CB

Survival data graphed & saved on DISK 194. OAC1_87.IPG, OAC2_87. " , OAC3_87. "

OAC1-87.IAG 5AC1-87.IPG 10AC1-87.IPG

8/17/95 (Data From 3/24/95) - DISK 172

α-Chaconine + Folic acid experiment on PT1. Took info from disk (#172) + put into this notebook because I couldn't find ~~it~~ it in any other notebook. Used data + made a graph (saved on DISK 194) for Army. Saved as CHAC3_24.IPG.

SCAN#	CONCENTRATION	Intercept	
1	NC	4.76×10^4	4.62×10^4
2	NC	4.48×10^4	
4	5mg/L α-C	4.52×10^4	
5	10mg/L α-C	2.05×10^5	
6	50mg/L α-C	3.48×10^5	
8	10mg/L α-C, 5mg/L Folic Acid	2.17×10^5	2.73×10^5
13	10mg/L α-C, 5mg/L FA	3.28×10^5	
9	10mg/L α-C, 10mg/L FA	2.1×10^5	2.2×10^5
14	"	2.30×10^5	
10	10mg/L α-C, 20mg/L FA	3.78×10^4	3.45×10^5
15	"	3.12×10^5	
11	10mg/L α-C, 50mg/L FA	2.98×10^5	2.8×10^5
16	"	2.62×10^5	

8/21/95 TEST #2

FETAX experiment with α-Chaconine and Folic Acid on African embryos. Folic Acid stock was 100mg/L in fetax solution and α-Chaconine stock was 50mg/L (same as 8/7/95 experiment). All solutions were at pH 6.0-6.3. Started test @ 1:30pm + covered dishes with foil after test was setup.

DISH#	CONCENTRATION	ml α-C	ml FA	ml FIX	0	24	48	72	96
1	NC	0	0	10	25	25	25	25	19
2	NC	0	0	10	25	25	25	22	19
3	NC	0	0	10	25	25	25	25	19
4	1mg/L FA, 0 α-C	0	0.1	9.9	25	25	25	24	22
5	" "	0	0.1	9.9	25	25	24	23	22

SISH	CONCENTRATION	ml αC	ml FA	ml FTX	0	24	48	72	96
6	2.5mg/L FA, 0 αC	0	0.25	9.75	25	25	25	25	25
7	" "	0	0.25	9.75	25	25	25	25	23
8	5mg/L FA, 0 αC	0	0.5	9.5	25	25	25	25	23
9	" "	0	0.5	9.5	25	25	25	24	24
10	10mg/L FA, 0 αC	0	1	9	25	25	24	24	23
11	" "	0	1	9	25	25	25	25	22
12	20mg/L FA, 0 αC	0	2	8	25	25	25	25	24
13	" "	0	2	8	25	25	24	24	19
14	50mg/L FA, 0 αC	0	5	5	25	25	25	22	22
15	" "	0	5	5	25	25	25	25	24
16	0 FA, 2.5mg/L αC	0.5	0	9.5	25	25	24	23	22
17	" "	0.5	0	9.5	25	25	25	23	~~23~~ 22
18	" "	0.5	0	9.5	25	25	25	24	24
19	1mg/L FA, 2.5mg/L αC	0.5	0.1	9.4	25	25	24	23	23
20	" "	0.5	0.1	9.4	25	25	25	25	25
21	" "	0.5	0.1	9.4	25	25	25	23	20
22	2.5mg/L FA, 2.5mg/L αC	0.5	0.25	9.25	25	25	25	25	24
23	" "	0.5	0.25	9.25	25	25	25	25	24
24	" "	0.5	025	9.25	25	25	25	25	25
25	5mg/L FA, 2.5mg/L αC	0.5	0.5	9	25	25	25	25	25
26	" "	0.5	0.5	9	25	25	25	24	24
27	" "	0.5	0.5	9	25	25	25	24	24
28	10mg/L FA, 2.5mg/L αC	0.5	1	8.5	25	25	25	25	23
29	" "	0.5	1	8.5	25	25	24	23	21
30	" "	0.5	1	8.5	25	25	25	24	23
31	20mg/L FA, 2.5mg/L αC	0.5	2	7.5	25	25	24	24	23
32	" "	0.5	2	7.5	25	25	24	24	23
33	" "	0.5	2	7.5	25	24	24	23	20
34	50mg/L FA, 2.5mg/L αC	0.5	5	4.5	25	25	25	24	21
35	" "	0.5	5	4.5	25	25	22	22	21
36	" "	0.5	5	4.5	25	25	25	24	24
37	0mg/L FA, 5mg/L αC	1	0	9	25	25	24	22	20
38	" "	1	0	9	25	25	23	21	19
39	" "	1	0	9	25	25	25	24	20
40	1mg/L FA, 5mg/L αC	1	0.1	8.9	25	25	23	21	18
41	" "	1	0.1	8.9	25	24	24	24	22
42	" "	1	0.1	8.9	25	24	23	21	19
43	2.5mg/L FA, 5mg/L αC	1	0.25	8.75	25	25	24	21	17
44	" "	1	0.25	8.75	25	25	24	23	22

OBS	CONCENTRATION	mL/C	ml FA	ml FTX	0	24	48	72	96
45	2.5mg/L FA, 5mg/L aC	1	0.25	8.75	25	25	22	20	16
46	5mg/L FA, 5mg/L aC	1	6.5	8.5	25	25	23	23	20
47	" "	1	0.5	8.5	25	25	25	23	21
48	" "	1	0.5	8.5	25	25	24	23	16
49	10mg/L FA, 5mg/L aC	1	1	8	25	25	21	20	17
50	" "	1	1	8	25	25	23	20	16
51	" "	1	1	8	25	25	22	17	10
52	20mg/L FA, 5mg/L aC	1	2	7	25	25	22	22	20
53	" "	1	2	7	25	24	24	20	14
54	" "	1	2	7	25	25	22	22	20
55	50mg/L FA, 5mg/L aC	1	5	4	25	25	25	24	23
56	" "	1	5	4	25	25	23	23	21
57	" "	1	5	4	25	25	24	22	18
58	0 FA, 10mg/L aC	2	0	8	25	18	5	3	0
59	" "	2	0	8	25	3	0	0	0
60	1mg/L FA, 10mg/L aC	2	0.1	7.9	25	7	0	0	0
61	" "	2	0.1	7.9	25	7	0	0	0
62	2.5mg/L FA, 10mg/L aC	2	0.25	7.75	25	2	0	0	0
63	" "	2	0.25	7.75	25	5	1	0	0
64	5mg/L FA, 10mg/L aC	2	0.5	7.5	25	9	0	0	0
65	" "	2	0.5	7.5	25	9	1	1	0
66	10mg/L FA, 10mg/L aC	2	1	7	25	4	0	0	0
67	" "	2	1	7	25	7	0	0	0
68	20mg/L FA, 10mg/L aC	2	2	6	25	5	0	0	0
69	" "	2	2	6	25	5	0	0	0
70	50mg/L FA, 10mg/L aC	2	5	3	25	14	7	5	0
71	" "	2	5	3	25	13	1	1	0

Put in 3% formalin on Day 4 (96 hour).
Digitized + scored. Didn't like this data too much.
(the malformation data)

Test #2 survival data is on page 32.

Spreadsheets saved as ACFA821.WQ1
ACFA821A.WQ1
ACFA821B.WQ1
ACFA821C.WQ1
} DISK 194

8/29/95 TEST #3 (check page 17 for data)

FETAX experiment with α-Chaconine and Folic acid on African embryos. Folic Acid stock was 100mg/L in fetax solution and α-Chaconine stock was 50mg/L in fetax solution. All solutions were at pH 6.0-6.3. Started test at 11:30am & covered dish with foil.

#	CONCENTRATION	ml α-C	ml FA	ml FETX	0	24	48	72	96
1	NC	0	0	10	25	25	25	25	2
2	NC	0	0	10	25	23	21	20	1
3	NC	0	0	10	25	25	23	22	2
4	α-C, 1mg/L FA	0	0.1	9.9	25	25	25	25	2
5	"	0	0.1	9.9	25	24	21	21	2
6	"	0	0.1	9.9	25	23	23	22	2
7	α-C, 2.5mg/L FA	0	0.25	9.75	25	25	22	22	2
8	"	0	0.25	9.75	25	24	23	22	2
9	"	0	0.25	9.75	25	25	23	23	2
10	α-C, 5mg/L FA	0	0.5	9.5	25	23	22	21	2
11	"	0	0.5	9.5	25	25	25	25	2
12	"	0	0.5	9.5	25	23	23	22	2
13	α-C, 10mg/L FA	0	1	9	25	24	24	24	2
14	"	0	1	9	25	24	22	22	22
15	"	0	1	9	25	25	25	25	25
16	α-C, 20mg/L FA	0	2	8	25	23	23	23	23
17	"	0	2	8	25	24.5	24.5	24.5	25
18	"	0	2	8	25	25	25	25	25
19	α-C, 50mg/L FA	0	5	5	25	24	21	21	21
20	"	0	5	5	25	25	25	25	25
21	"	0	5	5	25	25	24	24	24
22	2.5mg/L α-C, 0 FA	0.5	0	9.5	25	24	22	22	20
23	"	0.5	0	9.5	25	24	23	21	20
24	"	0.5	0	9.5	25	23	22	22	22
25	2.5mg/L α-C, 1mg/L FA	0.5	0.1	9.4	25	24	22	22	21
26	"	0.5	0.1	9.4	25	24	24	24	24
27	"	0.5	0.1	9.4	25	23	19	19	19
28	2.5mg/L α-C, 2.5mg/L FA	0.5	0.25	9.25	25	24	22	22	22
29	"	0.5	0.25	9.25	25	24	24	22	22
30	"	0.5	0.25	9.25	25	24	24	23	23

DISH	CONCENTRTION	ml αC	ml FA	ml FFX	0	24	48	72	96
31	2.5mg/L αC, 5mg/L FA	0.5	0.5	9	25	24	22	22	22
32	"	0.5	0.5	9	25	24	22	22	22
33	"	0.5	0.5	9	25	23	22	22	22
34	2.5mg/L αC, 10mg/L FA	0.5	1	8.5	25	25	24	24	24
35	"	0.5	1	8.5	25	23	23	23	23
36	"	0.5	1	8.5	25	23	22	21	21
37	2.5mg/L αC, 20mg/L FA	0.5	2	7.5	25	23	23	23	22
38	"	0.5	2	7.5	25	25	23	21	20
39	"	0.5	2	7.5	25	24	21	20	20
40	2.5mg/L αC, 50mg/L FA	0.5	5	4.5	25	24	23	22	22
41	"	0.5	5	4.5	25	24	23	22	22
42	"	0.5	5	4.5	25	24	24	24	24
43	5mg/L αC, 0 FA	1	0	9	25	24	15	9	7
44	"	1	0	9	25	24	17	13	13
45	"	1	0	9	25	25	20	18	18
46	5mg/L αC, 1mg/L FA	1	0.1	8.9	25	25	22	14	14
47	"	1	0.1	8.9	25	25	24	17	13
48	"	1	0.1	8.9	25	25	23	23	23
49	5mg/L αC, 2.5mg/L FA	1	0.25	8.75	25	24	21	20	20
50	"	1	0.25	8.75	25	24	20	13	11
51	"	1	0.25	8.75	25	23	22	16	15
52	5mg/L αC, 5mg/L FA	1	0.5	8.5	25	25	23	15	13
53	"	1	0.5	8.5	25	25	19	14	14
54	"	1	0.5	8.5	25	24	21	18	17
55	5mg/L αC, 10mg/L FA	1	1	8	25	25	16	15	11
56	"	1	1	8	25	24	17	13	13
57	"	1	1	8	25	24	21	16	16
58	5mg/L αC, 20mg/L FA	1	2	7	25	23	17	17	17
59	"	1	2	7	25	23	20	16	16
60	"	1	2	7	25	21	17	14	14
61	5mg/L αC, 50mg/L FA	1	5	4	25	24	21	21	21
62	"	1	5	4	25	25	23	22	22
63	"	1	5	4	25	25	21	19	18
64	10mg/L αC, 0 FA	2	0	8	25	6	0	0	0
65	"	2	0	8	25	11	0	0	0
66	"	2	0	8	25	16	0	0	0
67	10mg/L αC, 1mg/L FA	2	0.1	7.9	25	1	0	0	0
68	"	2	0.1	7.9	25	0	0	0	0
69	"	2	0.1	7.9	25	19	0	0	0

DISH	CONCENTRATION	ml dC	ml FA	ml FX	0	24	48	72	96
70	10mg/L dC, 2.5mg/L FA	2	0.25	7.75	25	1	0	0	0
71	"	2	0.25	7.75	25	3	0	0	0
72	"	2	0.25	7.75	25	12	0	0	0
73	10mg/L dC, 5mg/L FA	2	0.5	7.5	25	4	0	0	0
74	"	2	0.5	7.5	25	11	0	0	0
75	"	2	0.5	7.5	25	19	0	0	0
76	10mg/L dC, 10mg/L FA	2	1	7	25	9	0	0	0
77	"	2	1	7	25	7	0	0	0
78	"	2	1	7	25	13	0	0	0
79	10mg/L dC, 20mg/L FA	2	2	6	25	4	0	0	0
80	"	2	2	6	25	12	0	0	0
81	"	2	2	6	25	20	1	0	0
82	10mg/L dC, 50mg/L FA	2	5	3	25	21	14	8	7
83	"	2	5	3	25	22	2	2	0
84	"	2	5	3	25	23	0	0	0

Put in 3% formalin on last day (9/2/95)
Digitized + scored also. Made into spreadsheets + saved on DBK 194 as AC-FA-1.WQ1 AC-FA-3.WQ1
AC-FA-2.WQ1 AC-FA-4.WQ1

9/16/95

PTI experiment with Vinblastine on albino embryos (50mg/L Vinblastine stock.)
D13K #198 - 30 min. incubation, 20µl di-4

SCAN	CONCENTRATION	intercepts	Avg
1-4	NC	5.96E4, 5.13E4, 6.49E4, 5.44E4	(5.76E4)
5-8	50mg/L Vinblastine	5.91E4, 5.24E4, 5.43E4, 5.56E4	(5.54E4)
9-12	25mg/L Vinblastine	5.72E4, 5.72E4, 5.82E4, 5.60E4	(5.72E4)
13-16	10mg/L "	4.66E4, 5.07E4, 5.20E4, 5.55E4	(5.12E4)
17-20	6.25mg/L "	5.36E4, 5.66E4, 5.94E4, 6.20E4	(5.79E4)
21-24	3.125mg/L "	5.43E4, 4.95E4, 5.87E4, 5.27E4	(5.38E4)
25	52mg/L Chaconine	4.79E4	

concentration	% of Control	Conc	% of Contr
50mg/L Vinblastine	96.2	3.125mg/L	93.4
25mg/L "	99.3		
10mg/L "	88.9		
6.25mg/L "	101		

graphed (using inverse) on D13\C198--Vinb9_6.IPC

9/19/95 DISK 194

graphs saved as 0ACFA829.IPG 2ACFA829.IPG 5ACFA829.IPG 1ACFA829.IPG

Data saved as: 0AC1829.GP 0AC2829.GP etc...

Calculating % survival with α-Chaconine & Folic Acid data from test #3 (8/29/95) - % Survival

DAY 1	0 mg/L αC	2.5 mg/L αC	5 mg/L αC	10 mg/L αC
0 mg/L FA	97.3%	94.67%	97.3%	44%
1 mg/L FA	96%	94.67%	100%	26.67%
2.5 mg/L FA	98.67%	96%	94.67%	21.3%
5 mg/L FA	94.67%	94.67%	98.67%	45.3%
10 mg/L FA	97.3%	94.67%	97.3%	38.67%
20 mg/L FA	97.3%	96%	89.3%	48%
50 mg/L FA	98.67%	96%	98.67%	88%
DAY 2				
0 FA	92%	89.3%	69.3%	0%
1 "	92%	86.67%	92%	0%
2.5 "	90.67%	93.3%	84%	0%
5 "	93.3%	88%	84%	6%
10 "	94.67%	92%	72%	0%
20 "	97.3%	89.3%	72%	1.3%
50 "	93.3%	93.3%	86.67%	21.3%
DAY 3				
0 FA	89.3%	86.67%	53.3%	0%
1 "	90.67%	86.67%	72%	0%
2.5 "	89.3%	89.3%	65.3%	0%
5 "	90.67%	88%	62.67%	0%
10 "	94.67%	90.67%	58.67%	0%
20 "	97.3%	85.3%	62.67%	0%
50 "	93.3%	90.67%	82.67%	13.3%
DAY 4				
0 FA	86.67%	82.67%	50.67%	0%
1 "	89.3%	85.3%	66.67%	0%
2.5 "	89.3%	89.3%	61.3%	0%
5 "	90.67%	88%	58.67%	0%
10 "	94.67%	90.67%	53.3%	0%
20 "	97.3%	82.67%	62.67%	0%
50 "	93.3%	90.67%	81.3%	9.3%

* Look at 11/28/95 for info on more graphs from these 2 experiments.

9/19/95 continued

graphs saved as: 0ACFA87.IPG, 2ACFA87.IPG, 5ACFA87.IPG, 10ACFA87.IPG
data saved as: 0AC187.GP, 0AC287.GP etc...

Calculating % survival w/ α-Chac + Folic Acid
data from test #1 (8/7/95)

DAY1	0mg/L αC	2.5mg/L αC	5mg/L αC	10mg/L αC
0mg/L FA	100%	98.7%	97.3%	0%
1mg/L FA	97.3%	100%	98.7%	0%
2.5mg/L FA	100%	97.3%	97.3%	0%
5 mg/L FA	100%	100%	97.3%	0%
10mg/L FA	100%	98.7%	96%	2.7%
20mg/L FA	100%	100%	97.3%	0%
50mg/L FA	98.7%	98.7%	97.3%	24%
DAY2				
0 FA	100%	62.7%	10.7%	0%
1 "	90.7%	69.3%	16%	0%
2.5 "	98.7%	70.7%	1.3%	0%
5 "	97.3%	72%	1.3%	0%
10 "	93.3%	60%	2.7%	0%
20 "	94.7%	64%	14.7%	0%
50 "	92%	74.7%	12%	0%
DAY3				
0 FA	97.3%	61.3%	8%	0%
1 "	88%	62.7%	12%	0%
2.5 "	97.3%	62.7%	0%	0%
5 "	94.7%	72%	1.3%	0%
10 "	89.3%	54.7%	0%	0%
20 "	92%	57.3%	6.7%	0%
50 "	90.7%	66.7%	5.3%	0%
DAY4				
0 FA	97.3%	45.3%	0%	0%
1 "	86.7%	57.3%	5.3%	0%
2.5 "	96%	50.7%	0%	0%
5 "	94.7%	66.7%	0%	0%
10 "	89.3%	49.3%	0%	0%
20 "	92%	52%	2.7%	0%
50 "	90.7%	62.7%	0%	0%

9/20/95

α-Chaconine experiment. Combined 3 α-Chac stocks together (each 50mg/L) to make 1 big α-Chac stock (50mg/L). Then from that stock I made a 5mg/L stock of α-Chac.
Dishes 1–8: added 20ul di-4 + nc. for ~~30 min.~~ 1 hour.

DISH	Concentration	ml αc	ml FTX	DAY 0 #	DAY 1 * all data in misty's book (page 7)
1	NC	0	10	25	added 20ul/40 di-4 ~~saved as scanA1 D3KF~~ NOT RUN
9	NC	0	10	25	* dyed + looked (20ul) at under scope
17	NC	0	10	25	
2	50mg/L αChac	10(50stock)	0	25	added 20ul/40 di-4 Saved as SCANA7. D3K1
10	50mg/L	10(")	0	25	* dyed (20ul) + looked at under scope (1.58E3) 254.8%
3	10mg/L	2(50stock)	8	25	added 20ul/40 di-4 saved as SCANA6. D3K198
11	10mg/L	2(")	8	25	* dyed (20ul) + looked at under scope (1.21E3) 195
4	5mg/L	10(5 stock)	0	25	added 20ul/40 di-4 Saved as SCANA5. D3K1
12	5mg/L	10(")	0	25	* dyed + looked at (1.28E3) 206.4%
18	5mg/L	10(")	0	25	
5	1mg/L	2(5stock)	98	25	added 20ul/40 saved as SCANA4. D3K198
13	1mg/L	2(")	98	25	* dyed + looked at (1.27E3) 204.8%
19	1mg/L	2(")	98	25	
6	0.5mg/L	1(5stock)	9	25	added 20ul/40 saved as SCANA3. D3K198
14	0.5mg/L	1(")	9	25	* dyed + looked at (1.59E3) 256.4%
20	0.5mg/L	1(")	9	25	
7	0.1mg/L	0.2 or 200ul	9.8	25	added 20ul/40 saved as SCANA2 D3K198
15	0.1mg/L	200ul	9.8	25	* dyed + looked at (911.26) 146.9%
21	0.1mg/L	200ul	9.8	25	
8	0.05mg/L	100ul	9.9	25	added 20ul/40 saved as SCANA1 D3K198
16	0.05mg/L	100ul	9.9	25	* dyed + looked at (620.13) 100%
22	0.05mg/L	100ul	9.9	25	

Graphed + saved on D1SK198 as AC9_20.D

10/11/95

Catallo experiment with TCAB + TCAOB.
TCAB is orange color and TCAOB is yellow color. Will be doing a FETAX experiment with African embryos. Weighed out 10mg of each + dissolved each in 20ml of DMSO. Made 20mg/L stock of each toxicant + also made NC stock of 4% DMSO

Albino Embryos vs. Alpha-Chac (Low Concentrations) 10

3:00pm

10.3.95 - Embryos approx 6hrs old at 2:00pm when test started (Stg 1-8)

Dish 1: NC for dishes 1-6
Dish 2: 0.5 mg/L α-chac for 26 hrs and di-8 for 30 min
Dish 3: 1.0 mg/L α-chac for 26 hrs and di-8 for 30 min
Dish 4: 2.5 mg/L α-chac for 26 hrs and di-8 for 30 min
Dish 5: 5.0 mg/L α-chac for 26 hrs and di-8 for 30 min
Dish 6: 10.0 mg/L α-chac for 26 hrs and di-8 for 30 min
Note: α-chac at pH 6.5

10.4.95 - Embryos divided from NC of 10.3.95 and placed in test dish (Stg 13)

Dish 7: NC for dishes 7-13
Dish 8: inc in ~~7.5~~ fetax for 26 hrs and ~~I~~ 0.25 mg/L α-chac for 25.5 hrs and di-8 for 30 min
Dish 9: inc in fetax for 26 hrs and 0.50 mg/L α-chac for 25.5 hrs and di-8 for 30 min
Dish 10: inc in fetax for 26 hrs and 1.0 mg/L α-chac for 25.5 hrs and di-8 for 30 min
Dish 11: inc in fetax for 26 hrs and 2.5 mg/L α-chac for 25.5 hrs and di-8 for 30 min
Dish 12: inc in fetax for 26 hrs and 5.0 mg/L α-chac for 25.5 hrs and di-8 for 30 min
Dish 13: inc in fetax for 26 hrs and 10.0 mg/L α-chac for 25.5 hrs and di-8 for 30 min

PHOTOS: 1000 ASA Film, 5X photo eyepiece, 10X objective, B filter, red filter

Picture 1 - Dish 1, 2 sec.,
* Picture 2 - Dish 1, 5 sec., same as picture 1
• picture 3 - Dish 1, 2 sec., different embryo from 1 & 2
picture 4 - Dish 1, 5 2 sec., same embryo as 3.
• picture 5 - Dish 2, 2 sec., different embryo
picture 6 - Dish 2, 5 sec, same as 5.
• picture 7 - Dish 2, 2 sec., different embryo
* picture 8 - Dish 2, 5 sec., same as 7.
• picture 9 - Dish 3, 2 sec, different
* picture 10 - Dish 3, 5 sec., same as 9
picture 11 - Dish 3, 2 sec., different embryo
picture 12 - Dish 3, 5 sec., same as 11
• picture 13 - Dish 4, 2 sec. different
picture 14 - Dish 4, 5 sec, same as 13
picture 15 - Dish 4, 2 sec, different embryo
* picture 16 - Dish 4, 5 sec., same as 15
picture 17 - Dish 5, 2 sec., different, mottled
* picture 18 - Dish 5, 5 sec., same as 17, mottled
picture 19 - Dish 5, 2 sec, different embryo, mottled
picture 20 - Dish 5, 5 sec. same as 19, mottled
picture 21 - Dish 6, 2 sec. different, mottled surface
picture 22 - Dish 6, 5 sec., same as 21, mottled surface
picture 23 - Dish 4, 2 sec., different embryo, mottled
* picture 24 - Dish 6, 5 sec., same as 23., mottled

C

Albino Embryos vs d-Chac (Low concentrations) 10.5.95

Day 2 of Experiment Started on 10.4.95 4:30pm

Photos: 1000 ASA, 10X objective, 5X photo eye piece, B-filter, red filter

Dish 1-6 incubated for 51 hrs in d-Chac & 25.5 hrs. in di-8.

Holding
Picture 1 - Dish 1, 1 sec., Freakin' embryo
picture 2 - " , 2 sec., same as 1
picture 3 - Dish 2, 1 sec., different embryo
picture 4 - Dish 2, 2 sec., same embryo as 3
picture 5 - Dish 3, 1 sec., different embryo
picture 6 - " , 2 sec., same as 5
picture 7 - Dish 4, 1 sec., different embryo
picture 8 - " , 2 sec., same as 7
picture 9 - Dish 5, 1 sec., different embryo
picture 10 - " , 2 sec., same as 9
picture 11 - Dish 6, 1 sec., different embryo
picture 12 - " , 2 sec., same as 11
picture 13 - Dish 7&8, 1 sec., different embryo, not very bright
picture 14 - " , 2 sec., same as 13, "
picture 15 - Dish 8&9, 1 sec., different embryo, "
picture 16 - " , 2 sec., same as 15 "
picture 17 - Dish 10, 1 sec., different embryo, a little brighter
picture 18 - " , 2 sec., same as 17 "
picture 19 - Dish 11, 1 sec., different embryo, "
picture 20 - " , 2 sec., same as 19 "
Load Feeding Holding
picture 21 - Dish 12, 1 sec., different embryo, brighter than pic
picture 22 - " , 2 sec, same as 21 "
picture 23 - Dish 13, 1 sec., different, swollen, non-unfolding, some degrading, "
picture 24 - Dish 13, 2 sec., same as 23, " " "
picture 25 - ~~Dish 7, 2 sec., different embryo~~ → no fluorescence
Dish 3, 2 sec., different embryo

Albino Embryos vs d-Chac (Low Concentration) 10.6.95

Day 3: 4:00pm

Dish 14 - 72+ hr old embryos inc in fetax for 48 hrs and 0.25 d-chac for 24 hr and di-8 for 30 min

Dish 15 - 72+ hr old embryos inc in fetax for 48 hrs and 0.50 mg/L d-chac for 24 hr and di-8 for 30 min

Dish 16 - 72+ hr old embryos inc in fetax for 48 hrs and 1.0 mg/L d-chac for 24 hr and di-8 for 30 min

Dish 17 - 72+ hr old embryos inc in fetax for 48 hrs and 2.5 mg/L d-chac for 24 hr and di-8 for 30 min

Dish 18 - 72+ hr old embryos inc in fetax for 48 hrs and 5.0 mg/L d-chac for 24 hr and di-8 for 30 min

Dish 19 - 72+ hr old embryos inc in fetax for 48 hrs and 10.0 mg/L d-chac. for 24 hr and di-8 for 30 min

Dish 7 - redyed with di-8 for 30 min
Dish 10 - redyed with di-8 for 30 min
Dish 11 - redyed with di-8 for 30 min

Photos: 1000 ASA, 10x objective, 5x photoeye piece, B-filter, red filter, photos taken in well plate

Picture 2 - Dish 7, 1 sec., embryo unfolded
Picture 3 - " 2 sec., same as 2
Picture 4 - Dish 10, 1 sec., embryo unfolded
Picture 5 - " 2 sec., same as 4
Picture 6 - Dish 11, 1 sec., embryo unfolded
Picture 7 - " 2 sec., same as 6

Nothing
Picture 8 - Dish 14, 1 sec, not very bright, embryo unfolded, "sickly", possibly dead
Picture 9 - Dish 14, 1 & sec, " " " " "
* Picture 10 - Dish 14, 2 sec, same embryo as 9
Picture 11 - Dish 15, 1 sec, embryo unfolded, normal looking
* Picture 12 - Dish 15, 2 sec, same embryo as 11, not very bright
Picture 13 - Dish 16, 1 sec, normal unfolded embryo, not as bright
* Picture 14 - Dish 16, 2 sec, same as 13.
Picture 15 - Dish 17, 1 sec, unfolded but degraded, maybe losing dye
* Picture 16 - Dish 17, 2 sec, same as 15
Picture 17 - Dish 18, 1 sec, broken up + degraded
* Picture 18 - Dish 18, 2 sec, same as 17
Picture 19 - Dish 19, 1 sec, degraded (all)
* Picture 20 - Dish 19, 2 sec, same as 19
Picture 21 - Dish 14, 5 sec, unFolded, normal
Picture 22 - Dish 15, 5 sec, unFolded, normal
Picture 23 - Dish 16, 5 sec, unFolded, normal
Picture 24 - Dish 17, 5 sec, unFolded, normal

FETAX. (10/12/95)

10/12/95

Set up FETAX experiment. Stocks were also made on this day. Experiment was set up at 4:30pm using 24 hour plus embryos, around stages 22-28.

DISH	CONC.	(4% DMSO) ml toxicant	(4% DMSO) ml FTX	SURVIVAL DAY 0	DAY 24	DAY 48	DAY 72	DAY 9
1	NC (4% DMSO FTx)	0	10	25	21			
2	NC (")	0	10	25	25			
3	10mg/L TCAOB (w/4% DMSO)	5 TCAOB	5	25	20			
4	"	5	5	25	22			
5	5mg/L TCAOB (")	2.5	7.5	25	23			
6	"	2.5	7.5	25	20			
7	2.5mg/L TCAOB (")	1.25	8.75	25	25			
8	"	1.25	8.75	25	19			
9	1mg/L TCAOB (")	0.5	9.5	25	21			
10	"	0.5	9.5	25	22			
11	0.5mg/L TCAOB (")	0.25	9.75	25	23			
12	"	0.25	9.75	25	21			
13	0.25mg/L TCAOB (")	0.125	9.875	25	24			
14	"	0.125	9.875	25	~~24~~ 17			
15	0.1mg/L TCAOB (")	0.05	9.95	25	24			
16	"	0.05	9.95	25	25			
17	NC (4% DMSO FTx)	0	10	25	24			
18	NC (")	0	10	25	23			
19	10mg/L TCAB (w/4% DMSO)	5 TCAB	5	25	21			
20	"	5	5	25	19			
21	5mg/L TCAB (")	2.5	7.5	25	25			
22	"	2.5	7.5	25	20			
23	2.5mg/L TCAB (")	1.25	8.75	25	22			
24	"	1.25	8.75	25	24			
25	1mg/L TCAB (")	0.5	9.5	25	23			
26	"	0.5	9.5	25	25			
27	0.5mg/L TCAB (")	0.25	9.75	25	25			
28	"	0.25	9.75	25	23			

CONCEN.	(4% DMSO) ml toxicant	(4% DMSO) ml FTX	SURVIVAL DAY 0	DAY 24	DAY 48	DAY 72	DAY 96
0.25mg/L TCAB(")	0.125	9.875	25	25			
"	0.125	9.875	25	24			
0.1mg/L TCAB(")	0.05	9.95	25	24			
"	0.05	9.95	25	24			

saved spreadsheet as TCAB1012.XLS on D1SKZ1

Not renewed because DMSO seems to be killing all the embryos. On 48 hr. day, they were all "cheesed up" looking.

10/17/95 DATA From 7/14/95
α-Chac + Folic Acid. Could not find this data in anyones notebook so I'm entering it in mine. Scans on D3K188

graph & data saved on D3K188 as ACFA1_14.IRG

SCAN	CONC	Intercepts		% Control
1	NC	1.11×10^5	1.28E5	100%
2	NC	1.49×10^5		
3	NC	1.31×10^5		
32	NC	1.20×10^5		
22	505mg/L α Chac	7.16×10^5		~~178.8%~~ 559.4%
23	10mg/L α Chac	4.11×10^5		321.1%
24	5mg/L α Chac	1.39×10^5		108.6%
25	5mg/L αC, 5mg/L FA	1.22×10^5		95.3%
26	5mg/L αC, 10mg/L FA	1.22×10^5		95.3%
27	5mg/L αC, 20mg/L FA	1.023×10^5		80.5%
33	10mg/L αC, 20mg/L FA	1.25×10^5		97.7%

10/20/95
Weighed out 10mg each of TCAB & TCAOB. Put each in a 50ml beaker & added 10ml of DMSO (Fluka - 41650) to each toxicant. Put on stirrer and let stir over the weekend.

10/25/95
Made a 50mg/L stock of α-Chaconine (from Mendel - new). Then put 20 embryos in different concentrations: NC, 50mg/L αC, 10mg/L, 5mg/L, 1mg/L

after scanned on computer. Saved on C:\.

10/26/95

Took neuralating embryos (albinos) ~24 hrs. old (~stage 13-15) and put them into these dilutions of α-Chac (made 10/25/95): NC, 0.1mg/L αC, 1 mg/L αC, 2mg/L αC + 5mg/L α Chac. Seven (7) embryos were put into each dish.

From a 50mg/L stock of α Chaconine:

		ml αC	ml FTX
5 2mg/L	=>	1ml	9ml
2mg/L	=>	0.4ml	9.6ml
1mg/L	=>	0.2ml	9.8ml
0.1mg/L	=>	0.02ml or 20ul	9.98ml

10/27/95

Took unFolded embryos (stage 26-27) from 10/25/95 layed and put them into another dilution set. These embryos have been in FETAX solution since 10/25/95. Put 7 embryos in each dish (same as 10/26/95 setup).

No Results on this test.

10/31/95

Made 10mg/L Acrylamide stock solution for FETAX experiment. Set up the following dishes: (pH=6.85) @ 1:30 pm.

DISH	CONCEN.	DAY0	DAY1	DAY2	DAY3	DAY4
1	NC	20	20	20	20	20
2	NC	20	20	19	19	19
3	10mg/L	20	19	19	19	19

CONCEN	DAY0	DAY1	DAY2	DAY3	DAY4
10mg/L	20	20	19	19	19
5mg/L	20	20	19	19	19
5mg/L	20	19	19	19	14
2.5mg/L	20	20	20	20	20
2.5mg/L	20	17	17	17	17
1mg/L	20	19	19	18	18
1mg/L	20	20	19	19	19
		↑ H_2O not changed	↑ fresh water	↑ fresh H_2O	↑ put in 3% Formalin

saved spreadsheet as ACRY1031.wri on DISK 214.

Set up dishes for PTI experiment using Acrylamide + Tomatine (50mg/L) - pH = 6.3. Used 20ml di-4, inc. for 30min. Albinos. Saved on disk #205.

SCAN	CONCEN		Intercept	% Control
1	NC		2.02 E4	NC = 2.11 E4 ave.
2	50mg/L Tomatine		6.41 E5	3038 %
3	25mg/L Tomatine	Used embryos from one pair	6.49 E5	3075.8%
4	10mg/L Tomatine		4.16 E5	1971.6 %
5	5mg/L Tomatine		3.94 E5	1867.3%
6	NC		2.20E4	
7	NC		3.39E4	~~186~~
8	10mg/L Acrylamide	97.5%	2.69E4	~~79.4%~~ 79.6%
9	5 mg/L "	Used embryos from 57.8	1.59E4	~~46.9%~~ 47%
10	2.5 mg/L "	one pair 137.5	3.34E4	98.8%
11	1 mg/L "	105.5	2.91E4	~~85.6%~~ 86.1%
12	NC	172.5	3.37E4	~~83.9%~~
13	10mg/L Acrylamide	Used	2.83E4	83.7%
14	5 mg/L Acrylamide	embryos from	2.76E4	~~81.9%~~ 81.7%
15	2.5mg/L "	both pairs	2.42E4	~~71.5%~~ 71.6%
16	1 mg/L "	mixed	2.73E4	~~81.0%~~ 80.8%

saved as ACRY1031.IPG on DISK 214

used ~~2.75~~ 3.38 E4 for NC for Acrylamide

11/7/95

Set up albinos and put them in α-Chaconine solutions, 50 mg/L (made 10/25/95): NC, 0.1, 1, 2, 5 mg/L. Put 6 embryos in each dish. Same as 10/26/95 exp.

11/8/95

Set up another dilution set of albino embryos from 11/7/95. NC, 5, 2, 1, .1 mg/L of α-Chaconine

	ml αC	ml FTX
5mg/L =>	1 ml	9 ml
2mg/L =>	0.4ml	9.6ml
1mg/L =>	0.2ml	9.8ml
0.1mg/L =>	20 µl	9.98ml

Put 6 embryos in each dish at stage 26-27.

From 11/7/95 setup:

5mg/L - 4 alive
2mg/L - 6 alive
1mg/L - 5 alive
0.1mg/L - 6 alive

11/9/95

Set up another dilution set of albinos from 11/7/95: NC, 5, 2, 1 + 0.1mg/L αChac. Put 6 embryos in each dish.

From 11/7/95 Setup:

5 mg/L - 0 alive
2 " - 6 "
1 " - 5 "
0.1 " - 6 "

From 11/8/95 setup:

5mg/L - 0 alive
2 " - 6 "
1 " - 6 "
0.1 " - 6 "

Injected embryo from 11/9/95 NC with Hamilton syringe & needle (into the brain). Found that the needle is too large to inject with the Fluoro-Gold into the brain area of the embryo. Couldn't keep the embryo from moving as we pushed the needle into it. (Had the embryo under anesthesia.) Embryo did live after trying to inject it.

Saved spreadsheet as AC11-7.WQ1 on DISK 214

11/10/95

From 11/7/95:

5mg/L α Chac - 0 alive
2mg/L " - 6 "
1 mg/L " - 5 "
0.1mg/L " - 6 "

From 11/8/95:

- 0 alive
- 6 "
- 6 "
- 6 "

From 11/9/95

- 0 alive
- 6 "
- 6 "
- 6 "

11/13/95

Set up Tomatine experiment (50mg/L stock made 10/30/95)

SAN	CONCEN.	ml toxicant	ml FTX	Intercept	% Contr. ave.
1	NC	0	10	1.38E5	=> 1.27E5
2	50mg/L tomatine	10	0	1.07E6	842.5%
3	25mg/L "	5	5	1.12E6	881.9%
4	10mg/L "	2	8	9.44E5	743.3%
5	5mg/L "	1	9	8.48E5	667.7%
6	50mg/L α Chac(PC)	10	0	1.04E6	
7	NC	0	10	1.27E5	
8	50mg/L tomatine	10	0	8.71E5	685.8%
9	25 "	5	5	8.79E5	692.1%
10	10 "	2	8	7.64E5	601.6%
11	5 "	1	9	7.20E5	566.9%
12	NC	0	10	1.16E6	
13	50mg/L tomatine	10	0	7.88E5	620.5%
14	25 "	5	5	8.13E5	640.2%
15	10 "	2	8	6.89E5	542.5%
16	5 "	1	9	4.57E5	359.8%

Saved on DISK 2056.
Used 20 ul di-4 and approx. 80 embryos in each dish.

Saved data on DISK 206 plus graphed, too.

11/10/95
Used 48 hr + old albino embryos for injection with Fluoro-Gold. Used the swivel micro minipulator with both types of dissecting scopes. Placed embryo in ~~the~~ a well slide + tested it out. Used the 31G needles + the 5cc glass syringe. Very little success due to the large syringe (air bubbles). Made a wax mold to put the embryo into to keep it from sliding away from the ~~st~~ needle. We had alot of trouble focusing on the embryo. We feel that the needle was too large to inject into the embryo. Embryos lived with visible side effects (brain damage, swimming irregular).

11/14/95
24 hr + old embryos used for injection of Fluoro-Gold. We used the 36 guage needle with the 1cc plastic syringe (worked better but needle hole ~~too~~ too large). When injected with needle, the embryos busted. Kept for observation. Tried both dissecting scopes again. The American Optical (grey) was better for a general injection (did not give us a real close look) but the other (black) was better for closer viewing.
We ordered some small needles + a smaller syringe + repeating dispenser from Alltech.

FETAX experiment with TCAB + TCAOB. Took 1ml of the EtOH solution (10mg TCAOB & TCAB in 10ml EtOH) and put into a 500ml vol. Flask. ~~Need~~ I then added 4ml of EtOH + then filled to the line with FETAX. Other attempts at mixed with FETAX failed - it all came out of solution. (Summary = 1ml sol. + 4ml EtOH + 495ml FETAX). Solution is still not good. TCAOB + TCAB have still precipitated out.

11/14/95 continued:

Next I will try taking 1/10 ml (100 µl) of the TCAOB + the TCAB solutions + putting them into FETAX. Add 4.9 ml of EtOH with this (so we have a 1% solution still) EtOH). This solution is a 2mg/L w/ 1% EtOH (TCAOB). The TCAB is also a 2mg/L solution w/ 1% EtOH. I put these solutions on the hot plates + put on low for overnight.

11/15/95

Setup FETAX experiment with Acrylamide. Used the Acrylamide stock (10mg/L - made 10/30/95) Embryos used were albinos at stage 26-27, approx. 48hr + old. Setup at 10:00 am.

DISH	CONCEN.	11/15/95 DAY 0	11/16/95 DAY 1	11/17/95 DAY 2	11/18/95 DAY 3	11/19/95 DAY 4	DAY 5
1	NC	25	24	24	21		0
2	NC	25	24	23	22		0
3	10mg/L Acrylamide	25	22	21	20		0
4	10mg/L "	25	25	24	24		17
5	5mg/L "	25	23	22	22		22
6	5mg/L "	25	24	24	24		0
7	2.5mg/L "	25	24	23	23		16
8	2.5mg/L "	25	22	22	21		15
9	1mg/L "	25	25	25	23		23
10	1mg/L "	25	25	25	25		25
			renewed	not renewed			

saved spreadsheet as ACRY1115.WQ1 on D3K2:4

Also setup a FETAX experiment with TCAOB + TCAB. (200 ug/L w/ 1% EtOH). TCAOB is pH = 6.8. TCAB pH = 6.6. Used embryos that were albinos at stage 26-27, approx. 48+hr. old. NC was made to be 1% EtOH FTX. The stock solutions of TCAOB + TCAB were of 1% EtOH also. Set up @ 12:00 pm.

	CONCEN.	11/15/95 DAY 0	11/16 DAY 1	11/17 DAY 2	11/18 DAY 3	11/19 DAY 4	DAY 5
	NC (1% EtOH)	25	23	22	22		0
	NC (")	25	23	23	22		0
	NC (")	25	25	24	21		0
	200 ug/L TCAOB (10 ml)	25	23	22	21		0
	"	25	23	23	22		22
	"	25	23	23	22		0
	100 ug/L TCAOB (5 ml)	25	24	24	21		17
	"	25	22	20	19		0+5
	"	25	25	24	23		0
	50 ug/L TCAOB (2.5 ml)	25	25	22	21		15
	"	25	24	24	22		0
	"	25	23	23	21		7
	25 ug/L TCAOB (1.25 ml)	25	23	22	22		14
	"	25	24	24	20		0
	"	25	25	24	23		0
17	NC (1% EtOH)	25	24	24	24		0
18	NC (")	25	25	25	23		0
19	NC (")	25	23	23	23		21
20	200 ug/L TCAB	25	24	23	22		17
21	"	25	23	23	22		19
22	"	25	21	19	19		16
23	100 ug/L TCAB	25	22	22	20		0
24	"	25	25	25	25		0
25	"	25	21	20	20		0
26	50 ug/L TCAB	25	24	23	22		0
27	"	25	25	24	24		22
28	"	25	24	24	24		16
29	25 ug/L TCAB	25	24	23	17		0
30	"	25	24	24	24		0
			renewed	not renewed			

saved this spreadsheet as TCAB1115.WK1 on 01/3/24

Made up 10 mg/L of Trimethyltin (TMT) (hydroxide) in FETAX.

Put TMT (10 mg/L) in 3 dishes w/ 25 embryos in each. Same embryos as previous experiment.

11/16 DAY 1 - 22, 25, 25, DAY 3 - 0, 0, 0

11/17 DAY 2 - 0, 19, 24

saved spreadsheet as TMT11-15.WK1 on 01/3/24

11/20/95

2-Chaconine and Folic Acid experiment on the PTI. Used the αChac. stock from 10/25/95 (50mg/L) and new Folic Acid (100mg/L). Used 20ul di-4 in each dish. DIBIC 207. Albinos

	CONTEN	ml αC	ml FA	Intercept
1	NC	0	0	2.8E5
2	NC	0	0	3.77E5
3	NC	0	0	2.63E5
4	PC(50mg/L αC)	10	0	1.23E6
5	1mg/L FA	0	0.1	3.41E5
6	2.5mg/L FA	0	0.25	3.47E5
7	5mg/L FA	0	0.5	3.9E5
8	10mg/L FA	0	1	3.08E5
9	0 FA, 2.5mg/L αC	0.5	0	2.86E5
10	1mg/L FA, 2.5mg/L αC	0.5	0.1	2.70E5
11	2.5mg/L FA, 2.5 "	0.5	0.25	2.63E5
12	5mg/L FA, 2.5 "	0.5	0.5	2.65E5
13	10mg/L FA, 25 "	0.5	1	2.5E5
14	0 FA, 5mg/L αC	1	0	3.02E5
15	1mg/L FA, 5mg/L αC	1	0.1	2.65E5
16	2.5mg/L FA, 5 "	1	0.25	2.63E5
17	5mg/L FA, 5 "	1	0.5	2.87E5
18	10mg/L FA, 5 "	1	1	3.3E5
19	0 FA, 10mg/L αC	2	0	3.8E5
20	1mg/L FA, 10 "	2	0.1	3.35E5
21	2.5mg/L FA, 10 "	2	0.25	3.28E5
22	5mg/L FA, 10 "	2	0.5	3.54E5
23	10mg/L FA, 10 "	2	1	3.75E5
24	1mg/L TMT			5.05E5
25	200mg/L TCAαB			3.65E5
26	200mg/L TCAB			4.45E5

Did not like this experiment

Setup TMT test (10mg/L)

	DAY0	DAY1	DAY2	DAY3
TMT 1 -	25	25	25	0
TMT 2 -	25	24	24	0

11/21/95

α-Chaconine and Folic Acid experiment on the PT1
Made new α Chac. stock today (50mg/L) & new
Folic Acid Stock (100mg/L). Used 20 ul di-4, in
each dish. Saved on DISK207. Used 55 embryos
for each run. Albinos

	CONCEN.	Intercept
1a	NC	3.3E5
2a	NC	3.8E5
3a	NC	2.75E5
4a	PC (10mg/L αC)	9.36E5
5a	1mg/L FA,	3.26E5
6a	2.5 FA,	3.44E5
7a	5 FA,	3.78E5
8a	10 FA,	3.77E5
9a	0 FA, 25mg/L αC	2.36E5
10a	1 FA "	4.32E5
11a	2.5 FA, "	3.68E5
12a	5 FA, "	3.33E5
13a	10 FA, "	2.38E5
14a	0 FA, 5mg/L αC	2.46E5
15a	1 FA, "	3.68E5
16a	2.5 FA, "	4.2E5
17a	5 FA, "	2.57E5
18a	10 FA, "	3.47E5
19a	0 FA, 10mg/L αC	6.84E5
20a	1 FA, "	6.26E5
21a	2.5 FA, "	1.04E6
22a	5 FA, "	8.71E5
23a	10 FA, "	7.96E5
24a	PC (50mg/L αC)	1.59E6

11/28/95

Made different graphs for the α-Chac / FA data
From 8/7/95 + 8/29/95. Saved on disk 194 as
(Combined the data) Combo0AC.IPG (Cathy.IPG, Cathy1.IPG,
Cathy2.IPG are individuals), Combo2AC.IPG, Combo5AC,
+ Combo1AC => Combos of all graphs of DAYS 1-4.

11/28/95 continued

Saved data sets as: DISK 194

Cathy.GP Cathy1.GP Cathy2.GP Cathy3.GP	0 mg/L d-Chaconine Days 1, 2, 3 + 4 Combined 8/7/95 + 8/29/95		Graph: Combo0AC.IPG
Cathy4.GP Cathy5.GP Cathy6.GP Cathy7.GP	Day 1 Day 2 Day 3 Day 4	2.5 mg/L d-Chaconine Combined data	Graph Combo2A
Cathy8.GP Cathy9.GP Cathy10.GP Cathy11.GP	Day 1 Day 2 Day 3 Day 4	5 mg/L d-Chac. Combined data	Graph: Combo5AC.IPG
Cathy12.GP Cathy13.GP Cathy14.GP Cathy15.GP	Day 1 Day 2 Day 3 Day 4	10 mg/L d-Chac Combined data	Graph: Combo10AC.IPG

From page 8 & page 14 in this book.

Do not like this data

9/29/95 DIBIL194

Calculating % survival with α-Chaconine + Folic Acid data from test #2 (8/21/95) — % Survival. Data on page 11!

DAY1	0 mg/L αC	2.5 mg/L αC	5 mg/L αC	10 mg/L αC
0 mg/L FA	100%	100%	100%	42% (2 dishes)
1 mg/L FA	(2 dishes) 100%	100%	97.3%	28% "
2.5 mg/L FA	↓ 100%	100%	100%	14% "
5 mg/L FA	100%	100%	100%	36% "
10 mg/L FA	100%	100%	100%	22% "
20 mg/L FA	100%	98.67%	98.67%	20% "
50 mg/L FA	100%	100%	100%	54% "

DAY2				(2 dishes)
0 mg/L FA	100%	98.67%	96%	10%
1 mg/L FA	(2 dishes) 98%	98.67%	93.3%	0%
2.5 mg/L FA	↓ 100%	100%	93.3%	2%
5 mg/L FA	100%	100%	96%	2%
10 mg/L FA	98%	98.67%	90.67%	0%
20 mg/L FA	98%	96%	90.67%	0%
50 mg/L FA	100%	96%	96%	16%

DAY3	~~96%~~	~~93.3%~~		(2 dishes)
0 mg/L FA	96%	~~94.67%~~ 93.3%	89.3%	6%
1 mg/L FA	(2 dishes) 94%	~~100%~~ 94.67%	88%	0%
2.5 mg/L FA	↓ 100%	~~97.3%~~ 100%	85.3%	0%
5 mg/L FA	98%	~~96%~~ 97.3%	92%	2%
10 mg/L FA	98%	96%	76%	0%
20 mg/L FA	98%	94.67%	85.3%	0%
50 mg/L FA	94%	93.3%	92%	12%

DAY4				(2 dishes)
0 mg/L FA	76%	90.67%	78.67%	0%
1 mg/L FA	(2 dishes) 88%	90.67%	78.67%	0%
2.5 mg/L FA	↓ 96%	97.3%	73.3%	0%
5 mg/L FA	94%	97.3%	76%	0%
10 mg/L FA	90%	89.3%	57.3%	0%
20 mg/L FA	86%	88%	72%	0%
50 mg/L FA	92%	88%	82.67%	0%

11/30/95 TEST #4 222

FETAX experiment with α-Chaconine + Folic acid on Albino embryos. Folic Acid stock was 100mg/L in FETAX solution + α-Chaconine stock was 52mg/L in FETAX solution made 11/30/95. All solutions were at pH 6.5-6.8. Started test at 2:30pm + covered dishes with Foil.

ISH	CONCENTRATION (mg/L)	0	24	48	72	96
1	0mg/L FA, 0mg/L α Chac	25	25	25	25	25
2	"	25	25	25	25	25
3	"	25	25	25	25	25
4	0.1mg/L FA, 0mg/L α C	25	25	25	25	25
5	"	25	25	25	25	25
6	"	25	25	25	25	25
7	0.25mg/L FA, 0mg/L α C	25	25	25	24	24
8	"	25	25	25	25	25
9	"	25	25	25	25	25
10	0.5mg/L FA, 0mg/L α C	25	25	25	25	25
11	"	25	25	25	25	25
12	"	25	25	25	25	25
13	1mg/L FA, 0mg/L α C	25	25	25	25	25
14	"	25	25	25	25	25
15	"	25	25	25	25	25
16	2mg/L FA, 0mg/L α C	25	25	25	25	25
17	"	25	25	25	25	25
18	"	25	25	25	25	25
19	5mg/L FA, 0mg/L α C	25	25	25	25	25
20	"	25	25	25	25	25
21	"	25	25	25	25	25
22	0mg/L FA, 2.5mg/L α C	25	24	22	~~14~~	11
23	"	25	25	25	19	11
24	"	25	25	25	20	1
25	1mg/L FA, 2.5mg/L α C	25	25	24	19	13
26	"	25	25	24	21	0
27	"	25	25	24	21	17
28	2.5mg/L FA, 2.5mg/L α C	25	25	23	~~22~~ ~~22~~ ~~25~~	~~25~~ 21
29	"	25	25	25	~~22~~ 23	23
30	"	25	25	24	15	15

DISH	CONCENTRATION	0	24	48	72	96
		~~25~~				
31	5mg/L FA, 2.5mg/L αC	25	25	25	17	8
32	"	25	25	25	18	14
33	"	25	25	24	21	19
34	10mg/L FA, 2.5mg/L αC	25	25	25	23	22
35	"	25	25	25	23	21
36	"	25	25	23	22	19
37	20mg/L FA, 2.5mg/L αC	25	25	25	22	16
38	"	25	25	24	23	23
39	"	25	25	24	22	19
40	50mg/L FA, 2.5mg/L αC	25	25	24	24	24
41	"	25	25	25	25	24
42	"	25	25	25	25	25
43	0 FA, 5mg/L αC	25	22	9	0	0
44	"	25	22	9	0	0
45	"	25	23	13	0	0
46	1mg/L FA, 5mg/L αC	25	25	16	1	0
47	"	25	22	9	0	0
48	"	25	23	8	1	0
49	2.5mg/L FA, 5mg/L αC	25	24	11	0	0
50	"	25	20	6	0	0
51	"	25	21	8	0	0
52	5mg/L FA, 5mg/L αC	25	19	7	0	0
53	"	25	25	6	0	0
54	"	25	24	5	0	0
55	10mg/L FA, 5mg/L αC	25	24	6	0	0
56	"	25	22	9	0	0
57	"	25	21	11	0	0
58	20mg/L FA, 5mg/L αC	25	24	12	0	0
59	"	25	25	14	1	0
60	"	25	25	14	4	0
61	50mg/L FA, 5mg/L αC	25	25	21	6	2
62	"	25	25	20	1	0
63	"	25	25	23	4	0
64	0 FA, 10mg/L αC	25	0	0	0	0
65	"	25	0	0	0	0
66	"	25	0	0	0	0
67	1mg/L FA, 10mg/L αC	25	0	0	0	0
68	"	25	0	0	0	0

DISK	CONCENTRATION	0	24	48	72	96
		~~25~~				
69	1mg/L FA, 10mg/L αC	25	0	0	0	0
70	2.5mg/L FA, 10mg/L αC	25	0	0	0	0
71	"	25	0	0	0	0
72	"	25	0	0	0	0
73	5mg/L FA, 10mg/L αC	25	0	0	0	0
74	"	25	0	0	0	0
75	"	25	0	0	0	0
76	10mg/L FA, 10mg/L αC	25	0	0	0	0
77	"	25	0	0	0	0
78	"	25	0	0	0	0
79	20mg/L FA, 10mg/L αC	25	0	0	0	0
80	"	25	0	0	0	0
81	"	25	0	0	0	0
82	50mg/L FA, 10mg/L αC	25	10	0	0	0
83	"	25	16	1	0	0
84	"	25	12	0	0	0

Put in 3% Formalin on last day.

Spreadsheets saved as AC11_30.WQ1 - Day 1
AC11_30A.WQ1 - Day 2
ON DISK #194 AC11_30B.WQ1 - Day 3
AC11_30C.WQ1 - Day 4

Saved data sets as; DISK 194

11_30A.GP
11_30B.GP
11_30C.GP
11_30D.GP
} 0mg/L α Chaconine Days 1, 2, 3, 4 } Graph: 0AC1130.IPG

11_30E.GP
11_30F.GP
11_30G.GP
11_30H.GP
} 2.5 mg/L α Chaconine Days 1, 2, 3, 4 } Graph: 2AC1130.IPG

30I.GP
30J.GP
30K.GP
30L.GP
} 5mgL αChac Days 1-4 } Graph: 5AC1130.IPG

30M.GP
30N.GP
30O.GP
30θ.GP
} 10mgL αChac Days 1-4 Day 3+4 are the same } Graph: 10AC1130.IPG

12/5/95

Took all survival data from TESTS 1, 3+4 and graphed them. Saved on DISK ~~207~~ 209
Graphs are saved as: ~~D1~~ AVGD1.IPG (average of all 3 tests - Day 1)

AVGD2.IPG (ave of all 3 tests - Day 2
AVG.D3.IPG
AVGD4.IPG

All the data was saved on DISK 209, also.
All the data was transformed (Y/A $A=.75$) + then averaged. Each transformed data set was saved + then each set was saved again after being averaged.

Made tables in WordPerfect 5.1 on DISK ~~207~~ 209.
Saved as TESTS134.WPM.

Test #5 was also put in tables in WP5.1. the new Filename is TEST1345.WPM.

On 12/12/95 - included test 5 in all the graphs.
Now saved as: AVGD1_A.IPG (average of Tests 1,3,4,5 - DAY1,
AVGD2_A.IPG
AVGD3_A.IPG
AVGD4_A.IPG
} DISK 209

2/5/95

Calculating % survival w/ αChaC + Folic Acid data from TEST #4 (11/30/95) - % SURVIVAL

DAY1	0 mg/L αC	2.5 mg/L αC	5 mg/L αC	10 mg/L αC
0 mg/L FA	100%	98.67%	89.3%	0%
1 "	100%	100%	93.3%	0%
2.5 "	100%	100%	86.67%	0%
5 "	100%	100%	90.67%	0%
10 "	100%	100%	89.3%	0%
20 "	100%	100%	98.67%	0%
50 "	100%	100%	100%	50.67

DAY2				
0 mg/L FA	100%	96%	42.67%	0%
1 "	100%	96%	44%	0%
2.5 "	100%	96%	33.3%	0%
5 "	100%	98.67%	24%	0%
10 "	100%	97.3%	34.67%	0%
20 "	100%	97.3%	53.3%	0%
50 "	100%	98.67%	85.3%	1.33%

DAY3				
0 mg/L FA	100%	70.67%	0%	0%
1 "	100%	81.3%	2.67%	0%
2.5 "	98.67%	84%	0%	0%
5 "	100%	74.67%	0%	0%
10 "	100%	90.67%	0%	0%
20 "	100%	89.3%	6.67%	0%
50 "	100%	98.67%	14.67%	0%

DAY4				
0 mg/L FA	100%	30.67%	0%	0%
1 "	100%	40%	0%	0%
2.5 "	98.67%	78.67%	0%	0%
5 "	100%	54.67%	0%	0%
10 "	100%	82.67%	0%	0%
20 "	100%	77.3%	0%	0%
50 "	100%	97.3%	2.67%	0%

12/5/95 continued

Set up a small test with TMT using Stovers embryos.

saved spreadsheet as TMT12_5.WQ1 on Disk 214

	CONCEN.	0	24	48	72	96
1	NC	15	14	14	14	14
2	NC	15	14	14	14	14
3	NC	15	15	15	15	15
4	NC	15	14	14	13	13
5	1mg/L TMT	15	15	15	15*	14*
6	1mg/L TMT	15	15	14	14*	13*
7	1mg/L TMT	15	15	15	15*	15*
8	1mg/L TMT	15	15	14	14*	13*
9	10mg/L TMT (PC)	15	15	0	0	0

* deformed

12/8/95 DISK 208 αChac vs Folic Acid - PTI run
αC - Stock (50mg/L) - 12/8/95 pH 6.3 - Used African

SCAN	CONCEN	mL αC	mL FA	Intercept
1	NC	0	0	1.95E5
2	PC (50mg/L)	10	0	1.166E6
3	1mg/L FA	0	.1	2.85E5
4	2.5 FA	0	.25	4.17E5
5	5	0	.5	3.3E5
6	10	0	1	3.3E5
7	DFA, 2.5αC	.5	0	4.4E5
8	2.5αFA,"	.5	.1	2.84E5
9	2.5FA,"	.5	.25	2.82E5
10	5FA,"	.5	.5	1.94E5
11	10 FA,"	.5	1	1.36E5
12	NC	0	0	2.2E5
13	NC	0	0	4.03E5
14	PC (10mg/L)	2	0	9.58E5
15	0 FA, 5αC	1	0	5.62E5
16	1 " , "	1	.1	6.13E5
17	2.5" , "	1	.25	5.68E5
18	5" , "	1	.5	7.66E5
19	10" , "	1	1	7.51E5

	Concen	mlaC	mlFA	Intercept
0FA,	10aC	2	0	1.06E6
1 "	"	2	.1	8.05E5
2.5 "	"	2	.125	1.20E6
5 "	"	2	.5	8.85E5
20 "	"	2	1	8.9E5
	NC	0	0	

Continued on page 42. Scans 25-33

12/8/95 TEST #5 (Africans)
FETAX experiment with a-Chac + Folic Acid on Normal embryos. Folic Acid stock was 100mg/L in FETAX solution + a-Chaeonine stock was 50mg/L in FETAX solution made 12/8/95. All solutions were 6.3-6.4. Started test at 3:30pm Covered dishes with Foil.

DSH	CONCENTRATION (mg/L)	0	24	48	72	96
1	NC	25	25	25	25	25
2	NC	25	25	25	25	25
3	NC	25	25	25	25	25
4	0 mg/L FA, 0 mg/L aC	25	25	24	24	24
5	"	25	25	25	25	25
6	"	25	25	25	25	25
7	2.5 mg/L FA, 0 mg/L aC	25	25	25	25	25
8	"	25	25	25	25	25
9	"	25	25	25	25	25
10	5 mg/L FA, 0 mg/L aC	25	25	25	25	25
11	"	25	25	25	25	25
12	"	25	25	25	25	25
13	10 mg/L FA, 0 mg/L aC	25	25	25	25	25
14	"	25	25	25	25	25
15	"	25	25	25	25	25
16	20 mg/L FA, 0 mg/L aC	25	25	25	25	25
17	"	25	25	25	25	25
18	"	25	25	25	25	25
19	50 mg/L FA, 0 mg/L aC	25	25	25	25	25
20	"	25	25	25	25	25

	CONCEN (mg/L)	0	24	48	72	96
1	50mg/L FA, 0mg/L aC	25	25	25	25	25
2	0mg/L FA, 2.5mg/L aC	25	25	3	3	0
3	"	25	25	13	6	3
4	"	25	25	3	0	0
5	1mg/L FA, 2.5mg/L aC	25	25	14	6	2
6	"	25	25	15	6	0
7	"	25	25	10	1	1
8	2.5mg/L FA, 2.5mg/L aC	25	25	16	11	2
9	"	25	25	19	16	10
10	"	25	25	24	5	1
11	5mg/L FA, 2.5mg/L aC	25	25	16	0	0
12	"	25	25	21	10	3
13	"	25	25	9	1	1
14	10mg/L FA, 2.5mg/L aC	25	25	20	10	0
15	"	25	25	22	14	5
16	"	25	24	17	14	1
17	20mg/L FA, 2.5mg/L aC	25	25	24	10	0
18	"	25	25	21	13	5
19	"	25	25	23	9	0
40	50mg/L FA, 2.5mg/L aC	25	25	24	22	21
41	"	25	25	24	23	23
42	"	25	25	24	24	24
43	0 FA, 5mg/L aC	25	23	0	0	0
44	"	25	23	0	0	0
45	"	25	24	0	0	0
46	1mg/L FA, 5mg/L aC	25	25	0	0	0
47	"	25	25	0	0	0
48	"	25	25	0	0	0
49	2.5mg/L FA, 5mg/L aC	25	25	0	0	0
50	"	25	25	0	0	0
51	"	25	24	0	0	0
52	5mg/L FA, 5mg/L aC	25	24	1	0	0
53	"	25	25	0	0	0
54	"	25	23	0	0	0
55	10mg/L FA, 5mg/L aC	25	25	0	0	0
56	"	25	25	0	0	0
57	"	25	25	0	0	0
58	20mg/L FA, 5mg/L aC	25	25	0	0	0
59	"	25	25	0	0	0

	CONCEN (mg/L)	0	24	48	72	96
70	20mg/L FA, 5mg/L aC	25	25	0	0	0
71	50mg/L FA, 5mg/L aC	25	25	0	0	0
72	"	~~25~~ 24	24	0	0	0
73	"	25	25	2	0	0
74	0 FA, 10mg/L aC	25	0	0	0	0
75	"	25	0	0	0	0
76	"	25	3	0	0	0
77	1mg/L FA, 10mg/L aC	25	8	0	0	0
78	"	25	0	0	0	0
79	"	25	0	0	0	0
80	2.5mg/L FA, 10mg/L aC	25	0	0	0	0
81	"	25	0	0	0	0
82	"	25	0	0	0	0
83	5mg/L FA, 10mg/L aC	25	0	0	0	0
84	"	25	0	0	0	0
85	"	25	0	0	0	0
86	10mg/L FA, 10mg/L aC	25	13	0	0	0
87	"	25	~~0~~ 5	0	0	0
88	"	25	0	0	0	0
89	20mg/L FA, 10mg/L aC	25	1	0	0	0
90	"	25	20	0	0	0
91	"	25	0	0	0	0
92	50mg/L FA, 10mg/L aC	25	20	0	0	0
93	"	25	13	0	0	0
94	"	25	15	0	0	0

Saved spreadsheets on Disk 208 as:
AC_FA1.WQ1
AC_FA2.WQ1
AC_FA3.WQ1
AC_FA4.WQ1

Put in 3% Formalin on last day.

Continued from page 39.

N	CONCEN	mL aC	mL FA	Intercept
25	20mg/L FA, 0 aC	0	82	3.26E5
26	50mg/L FA, 0 aC	50	5	4.72E5
27	20 " , 2.5mg/L aC	.5	2	2.61E5
28	50 " , "	.5	5	5.35E5
29	20 " , 5 aC	1	2	5.86E5
30	50 " , "	1	5	4.63E5
31	20 " , 10 aC	2	2	1.21E6
32	50 " , "	2	5	4.18E5
33	NC	0	0	6.33E5

12/12/95

Combined all data of all 4 aChac/FA trials (tests 1, 3, 4, 5) + made graphs.

Disk 209 saved as AVG-D1_A.IPG
AVG-D2_A.IPG
AVG-D3_A.IPG
AVG-D3_A.IPG

The data was saved as:

DAY 1
- 0mg/L AC → D1_0AC.GP
- 2.5mg/L AC → D1_2AC.GP
- 5mg/L AC → D1_5AC.
- 10mg/L AC → D1_1AC.

D2_0AC.
D2_2AC.
D2_5AC.
D2_1AC.
D3_0AC.
↓
etc.

data was Averaged + saved as:
AVD1_0AC.GP
AVD1_2AC
AVD1_5AC
AVD1_1AC
AVD2_0AC
AVD2_2AC
↓
etc.

Look at page 36 for more info.

12/12/95

Calculating % survival w/ αChac + Folic Acid data from 12/8/95 TEST #5

DAY 1	0 mg/L αC	2.5 mg/L αC	5.0 mg/L αC	10 mg/
0 mg/L FA	100%	100%	93.3%	4%
1 "	100%	100%	100%	10.7%
2.5 "	100%	100%	98.7%	0%
5 "	100%	100%	96%	0%
10 "	100%	98.7%	100%	24%
20 "	100%	100%	100%	28%
50 "	100%	100%	98.7%	64
DAY 2				
0 mg/L FA	100%	25.3%	0%	0%
1 "	98.7%	52%	0%	0%
2.5 "	100%	78.7%	0%	0%
5 "	100%	64%	1.3%	0%
10 "	100%	78.7%	0%	0%
20 "	100%	90.7%	0%	0%
50 "	100%	97.3%	2.7%	0%
DAY 3				
0 mg/L FA	100%	12%	0%	0%
1 "	98.7%	17.3%	0%	0%
2.5 "	100%	42.7%	0%	0%
5 "	100%	14.7%	0%	0%
10 "	100%	50.7%	0%	0%
20 "	100%	42.7%	0%	0%
50 "	100%	92%	0%	0%
	~~100~~			
DAY 4				
0 mg/L FA	100%	4%	0%	0%
1 "	98.7%	4%	0%	0%
2.5 "	100%	17.3%	0%	0%
5 "	100%	5.3%	0%	0%
10 "	100%	8%	0%	0%
20 "	100%	6.7%	0%	0%
50 "	100%	90.7%	0%	0%

12/13/95 DISK 210 - Albinos

Ran TMT experiment on PT1. Used 10mg/L stock of TMT made 11/15/95. pH = 6.4.

	CONCEN.	ml TMT	Mt.	
	NC	0	9.31E5	1.02 E6
	NC	0	1.11E6	
	1 mg/L TMT	1	9.81E5	96.2%
	"	1	1.00E6	98.0%
	0.5 mg/L TMT	.5	9.14E5	89.6%
	"	.5	9.54E5	93.5%
	0.2 mg/L TMT	.2 or 200 ul	8.62E5	84.5%
	"	.2 "	9.82E5	96.3%
	0.1 mg/L TMT	.1 or 100 ul	9.45E5	92.6%
	"	.1 "	9.93E5	97.4%
	0.05 mg/L TMT	.05 or 50 ul	1.03E6	101.0%
	"	.05 or 50 ul	1.05E6	103.0%
	0.01 mg/L TMT	.01 or 10 ul	9.99E5	97.9%
	"	.01 or 10 ul	9.87E5	96.8%

graph saved as TMT12_13.IPG on DISK 214

Set up FETAX test using TMT (11/15/95 - 10mg/L stock)
Test started at 9:30 am. ~ Stages 8-9.

DISH	CONCEN	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	NC	10	10	10	10	10	10
2	1 mg/L TMT	10	10	10	10	10	10*
3	0.5 mg/L "	10	10	10	10	10	10*
4	0.2 "	10	10	10	10	10	10
5	0.1 "	10	10	10	10	10	10
6	0.05 "	10	10	10	10	10	10
7	0.01 "	10	10	10	10	10	10

saved as TMT12_13.WQ1 DISK 214

* 3% form

12/14/95

Set up another FETAX test using TMT (same as above)
Test started at 8:30 am. ~ Stages 17-27.
Same embryos from 12/13/95.

DISH	CONCEN.	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
1	NC	10	10	10	10	10

12/14/95 Continued

	CONCEN.	DAY0	DAY1	DAY2	DAY3	DAY4
2	1 mg/L TMT	10	10	10	10	10 *
3	.5 "	10	10	10	9	8 *
4	.2 "	10	10	10	10	10
5	.1 "	10	10	10	10	10
6	.05 "	10	10	10	10	10
7	.01 "	10	10	10	10	10

saved as TMT12_14.WQ1 on DISK 214

↑ 3% formalin

12/14/95

SYSTAT PROBIT

~~Create data in Lotus~~

~~Import from Lotus (Survival Files) i.e. DAYONEVR~~

~~Create PROBIT Column~~

1. Go into SYSTAT
2. Go to File, Import/Export, Import, File, ESC. Type File name (B:\DAYONEVR.WKS), ENTER ↑ type (LOTUS)
3. Save, ESC type Filename
4. Double, ESC
5. GO!
6. ESC, ESC, ESC
7. Data, Edit, ENTER
8. ESC, type "USE "DAYONEVR"
9. ESC
10. Create new Column named PROBIT. ENTER
11. ESC, Save as TEMP, ENTER.
12. QUIT (type)
13. Data!, ENTER
14. type LET PROBIT = ASN(SQR(NUMBER/25)) ENTER
15. Save (type) DAYONEVR ENTER
16. Yes
17. Run
18. QUIT
19. Edit! (check for data)
20. ESC & "Save DAYONEVR"

12/15/95

Set up another FETAX test using TMT using same embryos from 12/13/95. ~ Stages 27-31. Test started @ 11:00am.

CONCEN	DAY0	DAY1	DAY2	DAY3	DAY4
NC	10	10	10	10	9
mg/L TMT	10	10	10	10	10 *
0.5 "	10	10	10	10	10 *
0.2 "	10	10	10	10	10
0.1 "	10	10	10	10	10
0.05 "	10	10	10	10	10
0.01 "	10	10	10	10	10

Saved as TMT12_15.WQ1 on DISK 214 ↑ 3% formalin

*All concentrations of 0.5mg/L + 1mg/L => deformed. Not renewed, just recorded.

12/18/95

Saved as TMT12_18.WQ1 DISK 214

Digitized 225 on disk saved as TMT12_18. PRN

Set up FETAX experiment with TMT and albino embryos. pH of TMT = 6.7, → 3% FORMALIN ON DAY 4 STB

DISH	CONCEN	DAY0	DAY1	DAY2	DAY3	DAY4
1	NC	25	25	25	25	24
2	1mg/L TMT	25	25	25	25	0 ~~0~~ ~~18~~
3	.5 "	25	25	25	25	~~2~~ 16 all dead
4	.2 "	25	25	24	24	~~2~~ ~~+~~ 22
5	.1 "	25	25	25	25	~~24~~ 23
6	.05 "	25	25	25	25	~~25~~ 23
7	.01 "	25	25	25	25	~~25~~ 22
8	NC	25	25	22	22	~~25~~ 21
9	1mg/L TMT	25	25	25	25	0
10	.5 "	25	24	22	22	14
11	.2 "	25	25	25	25	~~16~~ 25
12	.1 "	25	25	25	25	~~24~~ 24
13	.05 "	25	25	25	25	25
14	.01 "	25	25	25	25	24

12/18/95 (con't)

Set up PT1 experiment w/ TMT using albino embryos. Saved on D3IC 210.

CAN	CONCEN	Intercept	
1A	NC	4.98 E5	5.10
2A	NC	5.18 E5	~~5.08~~ E5
3A	PC (50mg/L aC - 11/~~15~~21/95)	8.58 E5	~~174.8%~~ 170.
4A	PC (10mg/L TMT - 11/15/95)	4.99 E5	~~98.2%~~ 96.
5A	1 mg/L TMT	4.83 E5	~~95.1%~~ 92.
6A	.5 "	4.98 E5	~~98.0%~~ 95
7A	.2 "	4.38 E5	~~86.2%~~ 8.4.
8A	.1 "	4.46 E5	~~87.8%~~ 85
9A	.05 "	4.97 E5	~~97.8%~~ 95
10A	.01 "	5.91 E5	113.7 ~~116.3%~~
11A	NC	5.16 E5	~~101.6%~~
12A	NC	5.46 E5	~~107.5%~~

Used ave 4 NC's.

Saved graph @ TMT12-18.IPG on D3IC214

12/19/95 400 speed slide film.

Incubated embryos from 12/18/95 in dilutions of TMT (NC, 1mg/L, .5, .2, .1, .05, .01 mg/L TMT) on 12/18/95. Put in 40 ul di-4 for 2 hours. (In 20ul di-4 for ~45 min. after that)

- pic 1 - 2 sec, NC
- pic 2 - 5 sec, NC
- pic 3 - 10 sec, NC
- pic 4 - 20 sec, NC

} same embryo

- pic 5 - 2 sec, NC
- pic 6 - 5 sec, NC
- pic 7 - 10 sec, NC
- pic 8 - 20 sec, NC

} same embryo

pic 9 – 2sec, 1mg/L TMT
pic 10 – 5 "
pic 11 – 10 sec, 1mg/L TMT } same embryo
pic 12 – 20 sec, 1mg/L TMT
pic 13 – 20 sec, 1mg/L TMT
pic 14 – 2sec, .5mg/L TMT
pic 15 – 5sec, .5mg/L TMT } same embryo
pic 16 – 10sec, .5mg/L TMT
pic 17 – 20sec, .5mg/L TMT
pic 18 – 2sec, .2mg/L TMT – different embryo
pic 19 – 5sec, .2mg/L TMT
pic 20 – 10sec, .2mg/L TMT } same embryo
pic 21 – 20sec, .2mg/L TMT
pic 22 – 2sec., .1mg/L TMT
pic 23 – 5sec., .1mg/L TMT } same embryo
pic 24 – 10sec., .1mg/L TMT – different embryo

Did not turn out → (all)

12/20/95

Take photos of embryos (albinos) in TMT experiment (from 12/19/95.) Used P1600 film (Kodak Ektachrome) from Helt Photography. These embryos have been in di-4 for 24 hours. Same embryos from 12/19/95. Shot 1sec photos because moving too much.

pic 1 – 1 sec, neg control
pic 2 – 2sec, " } same embryo (took out ~6ml H_2O from dish)
pic 3 – 5 sec, "
pic 4 – 1 sec, 1 mg/L TMT – different embryo
pic 5 – 1 sec, " – " " } (took out ~6ml H_2O from dish)
pic 6 – 1 sec, " – " "
pic 7 – 1 sec, 0.5 mg/L TMT – different embryo
pic 8 – 1 sec, " } same embryo } took out ~7ml H_2O from dish
pic 9 – 1 sec, "
pic 10 – 1 sec, 0.2 mg/L TMT
pic 11 – 1 sec, " } same embryo } took out ~7ml H_2O from dish
pic 12 – 1 sec, "

* All photos on 12/20/95 were taken when the embryo's eye was focused.

pic13 - 1sec, .1 mg/L TMT	- different embryo	Took out ~7 ml H_2O from this dish	
pic14 - 1sec, "	same embryo		
pic15 - 1sec, "	same embryo		
pic16 - 1sec, .05 mg/L TMT	same embryo	Took out ~7 ml H_2O from this dish	
pic17 - 1sec, "	same embryo		
pic18 - 1sec, "	- different embryo		
pic19 - 1sec, ~~.01 mg/L TMT~~ NC	same embryo	same dish as pics 1-3	
pic20 - 1sec, NC	same embryo		
pic21 - 1sec, NC	- different embryo		

(continued pg 52) →

Pictures 1-21 embryos have been in FETAX for 24+ hours. before taking the photos.

in TMT for 24 hours then in FETAX for the second 24+ hours. (Embryos from 12/18/95)

the next set of embryos are still embryos laid on 12/18/95 but have been in TMT for 48 hours+. they've been in 40 ml di-4 for 2 hours

* page 52 for pictures

12/21/95

Setup FETAX experiment using TCAOB + TCAB on albino embryos. Setup @ 2:00 pm. Both stock solutions were 200 ug/L in 1% EtOH.

DISH	CONCEN	DAY0	DAY1	DAY2	DAY3	DAY4
1	NC (1% EtOH)	25	25	0		
2	200 ug/L TCAOB	25	24	0		
3	100 ug/L TCAOB	25	25	0		
4	50 ug/L TCAOB	25	25	0		
5	25 ug/L TCAOB	25	24	0		
6	10 ug/L TCAOB	25	23	0		

DISH	CONCEN.	DAY0	DAY1	DAY2	DAY3	DAY4
1	NC (1% EtOH)	25	25	6		
2	200 ng/L TCAOB	25	24	0		
3	100	25	25	0		
4	50	25	25	0		
5	25	25	24	0		
6	10	25	24	0		
7	NC (1% EtOH)	25	25	0		
8	200 ng/L TCAB	25	24	0		
9	100	25	23	0		
10	50	25	25	0		
11	25	25	25	0		
12	10	25	25	0		
13	NC (1% EtOH)	25	25	0		
14	200 ng/L TCAB	25	24	0		
15	100	25	25	0		
16	50	25	24	0		
17	25	25	23	0		
18	10	25	25	6		

saved spreadsheet as TCAB1221.WQ1 on D3K214

Set up FETAX experiment using 3 dehydrated tomatoes (Tomatoes 1345-4 green, tomatoes BEBC cherry red + tomatoes BEBC cherry green). All stock solutions were 1 gram / 5100 ml of fetax. Started test at 5:30 pm.

DISH	CONCEN	DAY0	DAY1	DAY2	DAY3	DAY4
1	NC	25	25			
2	2g/L BEBC cherry red	25	0	0		
3	1g/L " "	25	0	0		
4	.5g/L " "	25	0	0		
5	.25g/L " "	25	0	0		
6	NC	25	25			
7	2g/L BEBC cherry green	25	0	0		
8	1g/L " "	25	0	0		

CONCEN.		DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
.5g/L	BEBC Cherry green	25	0	0		
.25g/L	" "	25	0	0		
	NC	25	23			
2g/L	1345-4 green	25	0	0		
1g/L		25	0	0		
.5g/L		25	0	0		
.25g/L		25	5	2	0	0

12-22-95 Made up fresh α-chac from A/F spkw Adj pH to ~ 3 and stirred w/heat. Elsey 24hr EMBROS, INCubated with 40 µL D1-4 AND FTX.

DISH	[ ] FTX	STOCK	25 EMBRYOS/DSH
1	0	10 ML	
2	1 MG/L TMT	9	1 ML } TMT STOCK @ 10 MG/L
3	0.5 MG/L	9.5	0.5 }
4	0.25 MG/L	9.75	0.25 }
5	0.1 MG/L	9.9	0.1 }
6	0	10	
7	5 MG/L	9	1 ML α-C stock 12-22-95 50 MG/L
8	10	8	2
9	2.5	9.5	0.5
10	1	~~9.75~~ 9.75	0.25
11	0.5	9.9	0.1

Then I setup the camera with P1600 slide film, using NIKON bank + 5X Photolens, I used a combination of 10X + 5X objectives. EMBRYOS were INCubated for 4 HRS IN 20 µl D14 stock

PTX #	[ ]	OBJ	TIME	LIGHT
1	0 (1)	10X	1/30 S	INC.
2	0	"	1 S	FL B/580 BARRIER
3	0	"	2 S	FL
4	0	5X	1 S	"
5	0	"	2 S	"
6	1 MG/L TMT (2)	10X	1/30 S	INC @ 5.5
7	"	"	1 S	FL B/580 * made into 4x6
8	"	"	2 S	" "
9	"	5X	1 S	" " (cont'd)-ON ON P 52

pic 22 - 1 sec, NC, in TMT for 1 hr 45 min. — same embryo
pic 23 - NC
pic 24 - NC
pic 25 - , 1 mg/L TMT — same embryo
pic 26 - "
pic 27 - " "
pic 28 - , .5 mg/L TMT — same embryo
pic 29 - "
pic 30 - "
pic 31 - , .2 mg/L TMT — same embryo
pic 32 - "
pic 33 - "
pic 34 - , .1 mg/L TMT — same embryo
pic 35 - "
pic 36 - "
pic 37 - .05 mg/L TMT — ~~same~~ different embryo

Pix did not turn out! However there are some IMAGES ON NEGATIVES. Had some pictures made.

Cont'd from PS1 TMT/α-C 2ND P1600 EXPT/24

PIX [ ]	OBJ	TIME	COND	
10	0.5 MG/L TMT (dish 3)	10X	1/30 S	INCANDESCENT
11	"	"	1 S	FL B/580 * made into 4x6
12	"	"	2 S	" "
13	"	5X	1 S	" "
14	0.25 TMT (4)	10X	1/30 S	INC.
15	"	"	1 S	FL B/580 * made into 4x6
16	"	"	2 S	" "
17	"	5X	1 S	" "
18	0.1 TMT (5)	10X	1/30 S	INC
19	"	"	1 S	FL B/580 * made into 4x6
20	2.5 MG/L α-C	10X	1/30 S	INC Exposed 3 hr to α-C + D14
21	"	"	1 S	FL B/580
22	"	"	2 S	FL " " - moved (?)
23				
24	2.5 MG/L α-C	5X	1 S	FL " "

Continued →

Cont'd 24 h expt JMT/X-C using P/600 film

PIX	[ ]	OBJ	TIME	SOURCE	
25	1 mg/L X-C (dish 10	10	1/30S	INC	
26	ON-PS1	~~#~~	~~1S~~	~~FC~~	
27	"	"	2S	FL moved.	
~~28~~		SX	1S		
28	0.5 mg/L X-C	10X	1/30S	LT	
29	dish 11	"	1S	FL	B/580
30	"	"	2S	"	"
31	"	SX	1S	"	"
32	NC (12 0)	10X	1/30S	LT	
33		10X	1S	FL	B/580 * made into 4x6
34		"	2S	"	
35		SX	1S	"	

12-27-95

Set up chicken EXPT by making up chicken Ringer's solution, Used recipe in Prosser

NaCl	150
$NaHCO_3$	20
KCl	5
$CaCl_2$	5
$MgCl_2$	2
Glucose	12

Saved on PTI computer under Lotus as chickRNA

Warmed thy to 37° C Set up diskette #212 for chicken data. Protocol + Procedure is on diskette

1-2-96 X-C vs FA, Came up with scheme to analyze FA data by regressing FA onto Percent survival. Do this by using DAYONE.VR on Disk 209 to SORT by AC, then FA, then TRIAL, then DISA OUT PUT This as PRN FILE with JUST AC=0 (Row 2-85)

1/9/96
Most of the slides from 12-24-95 (pages 51, 52, 53) turned out.

1/10/96

Experiment using albinos, α-chaconine & SBFI dye. Counted out 80 embryos in each dish that contained 10 ml of FETAX solution. there are 19 dishes. After added 80 embryos to each dish, I made up the SBFI, by adding 100 µl DMSO to each of 4 vials of SBFI. I waited until the SBFI was dissolved, then added 20 µl from a vial to each dish of embryos. Set the timer for 4 hours + let sit in the SBFI. Each dish is labeled accordingly: PC (50mg/L), 3-10mg/L αC, 3-NC, 3-0.1mg/L, 3-1mg/L, 3-2.5mg/L, 3-5mg/L αC. We also made 2 dishes: 1-NC + 1-50mg/L αC using di-4 ANEPPS to test the toxicity of the αChac. the α-Chac. was made 1/9/96 in 50mg/L concentration. After being incubated for 4 hours in SBFI, the water was switched out to the concentrations and incubated another 30 min. Saved data on Disk 11. Ran in u-cuvette on PTI. Left the emission wavelength @ 620nm. Then changed to 505nm for next set.

SAN	concen.	ml αC	ml FTX			Intercept.
1	NC (di-4)	0	10			3.10E5
2	50mg/L αC (di-4)	10	0			1.04E6
3	NC (SBFI)	0	10	emission λ of 620 (last scan)	620 EX1=300 EX2=400	1.27E6
4	50mg/L αC ↓	10	0			1.02E6
5	0.1mg/L αC	20µl	9.98ml			1.28E6
6	1mg/L αC	.2ml	9.8			1.14E6
7	2.5mg/L αC	.5	9.5			1.23E6
8	5mg/L αC	1	9			1.28E6
9	10mg/L αC	2	8			1.26E6
10	NC	0	10	505nm	505 EX1=380 EX2=340	5.51E5
11	0.1mg/L αC	20µl	9.98			4.75E5
12	1mg/L αC	.2	9.8			4.81E5
13	2.5mg/L αC	.5	9.5			4.96E5
14	5mg/L αC	1	9			4.90E5

SCAN	CONCEN	ml aC	ml FTX		Intercept
15	10mg/L aC	2	8	−505nm	?
16	NC	0	10	505nm	4.98E5
17	.1mg/L aC	20ul	9.98		5.35E5
18	1 mg/L aC	.2	9.8		5.96E5
19	2.5 mg/L aC	.5	9.5		4.81E5
20	5 mg/L aC	1	9		5.19E5
21	10 mg/L aC	2	8		4.57E5

* 505nm looks to be the best position for the emission monochromator.

1/11/96

From ~~12-27-95~~ 1-5-96, Chicken data: intercepts for each scan, 1A + 2A. DISK 213. Day #10
Spreadsheet data on DISK220. Page 88 - modifications

SCAN	DYE	2A intercept	1A intercept
IA1	Di-4	2.65 E4	-609.32
IA2		1.21 E4	-658.45
IA3		3.86 E4	-456.18
IA4		2.95E4	-524.20
IB1		3.79E4	-599.98
IB2		3.15E4	-526.79
IB3		4.29E4	-544.66
IB4		4.94E4	-494.92
IC1		9.29E3	-685.82
IC2		5.95E4	-438.62
IC3		4.17E4	-464.81
IC4		2.07E4	-543.12
IIA1		3.10E4	-436.51
IIA2		1.97E4	-536.64
IIA3		1.29E4	-457.57
IIA4		2.50E4	-381.00
IIB1		4.41E4	-357.03

1/11/96 continued

AN	DYE	2A int.	1A int.
IIB2	Di-4	1.00E5	-110.34
IIB3		1.03E4	-511.00
IIB4		4.18E4	-426.98
IIC1		4.92E4	-395.87
IIC2		4.55E4	-321.69
IIC3		2.58E4	-326.25
IIC4		3.36E4	-445.08
IIIA1		6.07E4	2.05E3
IIIA2		2.00E4	-658.80
IIIA3		2.89~~1.29~~E4	-626.22
IIIA4		1.19E4	-560.31
IIIB1		3.15E4	-549.52
IIIB2		1.21E4	-705.21
IIIB3		4.04E4	-507.39
IIIB4		5.45E4	-448.69
IIIC1		2.56E4	-670.53
IIIC2		2.90E4	-559.50
IIIC3		1.40E4	-629.95
~~IIIC4~~			
IA1	BCECF	1.34E5	4.21E3
IA2		1.54E5	4.33E3
IA3		1.52E5	4.19E3
IA4		1.19E5	4.05E3
IB1		1.13E5	4.20E3
IB2		1.22E5	4.10E3
IB3		8.49E4	3.88E3
IB4		7.05E4	3.47E3
IB5C1		1.03E5	3.74E3
IC2		8.69E4	3.87E3
IC3		9.98E4	3.49E3
IC4		1.06E5	4.13E3
IIA1		1.40E5	4.03E3
IIA2		6.10E4	3.23E3
IIA3		8.61E4	3.53E3
IIA4		1.07E5	3.88E3
IIB1		1.19E5	3.99E3
IIB2		1.00E5	3.86E3

1/11/96 continued

SAN	DYE	2A int.	1A int.
IIB3	BCECF	1.27E5	3.77E3
IIB4		1.12E5	3.75E3
IIC1		9.20E4	3.90E3
IIC2		7.93E4	3.55E3
IIC3		2.33E5	4.70E3
IIC4		1.18E5	4.27E3
IIIA1		1.13E5	3.68E3
IIIA2		1.21E5	3.71E3
IIIA3		1.22E5	4.04E3
IIIA4		7.43E4	3.54E3
IIIB1		4.89E4	3.22E3
IIIB2		1.52E5	3.90E3
IIIB3		1.21E5	4.21E3
IIIB4		1.21E5	4.00E3
IIIC1		1.64E5	3.74E3
IIIC2		1.48E5	3.67E3
IIIC3		7.01E4	3.42E3
IA1	SNAFL	1.60E6	6.22E5
IA2		1.82E6	6.26E5
IA3		1.88E6	8.91E5
IA4		1.04E6	3.85E5
IB1		1.76E6	7.12E5
IB2		1.54E6	5.85E5
IB3		1.84E6	6.08E5
IB4		1.32E6	3.57E5
IC1		2.09E6	8.16E5
IC2		1.61E6	5.94E5
IC3		7.49E5	2.16E5
IC4		1.43E6	5.43E5
IIA1		2.00E6	9.40E5
IIA2		1.35E6	4.18E5
IIA3		1.25E6	3.80E5
IIA4		1.87E6	8.31E5
IIB1		9.86E5	3.51E5
IIB2		1.08E6	3.81E5
IIB3		1.0[illegible]E6	[illegible]

1/11/96 Continued

SAN	DYE	2A int.	1A int.
IIB4	SNAFL	1.63E6	6.20E5
IIC1		1.50E6	4.31E5
IIC2		1.55E6	6.43E5
IIC3		1.82E6	8.34E5
IIC4		1.85E6	8.37E5
IIIA1		1.09E6	3.80E5
IIIA2		2.00E6	1.02E6
IIIA3		2.67E6	1.14E6
IIIA4		1.78E6	6.21E5
IIIB1		1.47E6	4.68E5
IIIB2		1.78E6	8.43E5
IIIB3		1.72E6	6.43E5
IIIB4		1.08E6	3.41E5
IIIC1		2.07E6	1.86E6
IIIC2		1.77E6	8.50E5
IIIC3		1.74E6	5.94E5
IA1	FLUO	1.04E5	3.57E3
IA2		1.62E5	3.78E3
IA3		4.62E4	3.59E3
IA4		2.31E4	3.13E3
IB1		5.62E4	3.23E3
IA6 B2		4.74E4	2.94E3
IA7 B3		9.31E4	3.27E3
IA8 B4		6.99E4	3.14E3
IA9 C1		1.71E5	3.62E3
IC2		5.39E4	3.31E3
IC3		2.17E4	2.83E3
IC4		5.61E4	3.58E3
IIA1		5.91E4	3.32E3
IIA2		4.85E4	3.08E3
IIA3		1.07E5	3.43E3
IIA4		1.32E5	3.58E3
IIB1		3.86E4	3.22E3
IIB2		2.55E4	2.92E3
IIB3		6.09E4	3.00E3
IIB4		5.16E4	3.11E3

SCAN	DYE	2A int.	1A int.
IIC1	FLUO	7.39E4	3.24E3
IIC2		2.17E5	3.84E3
IIC3		2.56E5	4.19E3
IICA		3.49E4	3.58E3
IIB1		1.54E4	2.78E3
IIA2		6.27E4	3.31E3
IIA3		1.05E5	3.71E3
IIA4		9.95E4	3.49E3
IIB1		1.46E5	3.41E3
IIB2		1.78E5	4.02E3
IIB3		3.80E4	3.47E3
IIB4		1.63E4	2.92E3
IIC1		8.53E4	3.21E3
IIC2		7.32E4 7.33E4	3.07E3
IIC3		5.27E4	3.41E3
IA1	SBFI	7.63E3	8.34E4
IA2		8.82E3	1.03E5
IA3		7.13E3	9.00E4
IA4		1.06E4	8.31E4
IB1		8.08E3	8.97E4
IB2		8.20E3	8.36E4
IB3		5.00E3	6.53E4
IB4		5.99E3	7.41E4
IC1		7.35E3	1.01E5
IC2		1.78E4	1.15E5
IC3		7.16E3	7.15E4
IC4		8.30E3	9.34E4
IIA1		8.26E3	1.07E5
IIA2		6.84E3	6.92E4
IIA3		7.45E3	8.63E4
IIA4		1.45E4	1.79E5
IIB1		7.85E3	7.21E4
IIB2		7.31E3	7.06E4
IIB3		8.59E3	8.51E4
IIB4		8.25E3	9.98E4
IIC1		7.24E3	7.10E4

1/11/96 continued

SCAN	DYE	2A int	1A int
IIC2	SBFI	1.51E4	1.56E5
IIC3		1.32E4	1.52E5
IIC4		1.46E4	1.01E5
IIIA1		6.13E3	6.56E4
IIIA2		9.64E3	8.89E4
IIIA3		9.94E3	9.87E4
IIIA4		7.11E3	6.92E4
IIIB1		8.31E3	1.05E5
IIIB2		1.64E4	1.88E5
IIIB3		2.49E4	1.38E5
IIIB4		6.42E3	6.84E4
IIIC1		9.55E3	1.03E5
IIIC2		1.32E4	1.33E5
IIIC3	↓	6.91E3	7.02E4
IA1	PBFI	7.29E3	1.21E5
IA2		6.31E3	9.76E4
IA3		1.45E4	1.12E5
IA4		7.16E3	7.17E4
IB1		7.05E3	1.07E5
IB2		1.15E4	1.68E5
IB3		7.22E3	1.29E5
IB4		6.50E3	1.09E5
IC1		9.94E3	2.55E5
IC2		2.15E4	1.39E5
IC3		6.85E3	7.20E4
IC4		8.34E3	1.17E5
IIA1		8.67E3	1.62E5
IIA2		7.06E3	7.84E4
IIA3		5.01E3	8.59E4
IIA4		1.07E4	2.03E5
IIB1		2.05E4	1.42E5
IIB2		7.65E3	8.18E4
IIB3		1.28E4	2.14E5
IIB4		1.02E4	2.19E5
IIC1		6.67E3	7.93E4
IIC2		1.43E4	2.54E5
IIC3	↓	1.18E4	2.25E5

1/11/96 continued

SCAN	DYE	2A int.	1A int.
IIC4	PBFI	3.97E4	2.53E5
IIIA1		6.69E3	7.02E4
IIIA2		1.50E4	1.60E5
IIIA3		1.25E4	1.61E5
IIIA4		5.86E3	8.13E4
IIIB1		1.73E4	3.24E5
IIIB2		1.31E4	2.00E5
IIIB3		1.93E4	1.50E5
IIIB4		7.42E3	7.06E4
IIIC1		1.46E4	1.97E5
IIIC2		1.60E4	2.09E5
IIIC3	↓	~~1.69~~ 1.09E4	1.59E5

1/16/96

Ran chicken experiment. Check on DISK 215 under ROCHPROT. WPM + TIME. WPM. All scans are on DISK 215 also. Day ~~20~~ 20. Spreadsheet data on DISK 220. Page 88 - modifications

SCAN	DYE	2A int.	1A int.
IA1	di-4	3.59E5	4.44E3
IA2		1.86E5	2.74E3
IA3		4.54E5	4.81E3
IA4		8.25E5	7.58E3
IB1		4.26E5	4.51E3
IB2		1.93E5	7.41E3
IB3		4.13E5	4.66E3
IB4		4.72E5	4.33E3
IC1		2.87E5	4.46E3
IC2		6.67E5	6.81E3
IC3		IC3 → 4.24E5	5.45E3
IC4		IC4 → 3.04E5	4.51E3
IIA1		IIA1 → 1.78E5	4.38E3
IIA2		3.54E5	3.70E3
IIA3		2.86E5	3.05E3
IIA4		3.04E5	3.73E3
IIB1		3.27E5	1.10E4

[illegible]N	Dye ~~Di-4~~	2A int.	1A int.
IIB3	di-4	6.95E5	6.85E3
[illegible]4	↓	1.73E5	3.12E3
[illegible]		8.36E5	7.81E3
[illegible]C2		6.29E5	4.97E3
[illegible]3		1.06E6	7.25E3
[illegible]C4		3.86E5	7.15E3
[illegible]A1		4.17E5	3.08E3
[illegible]A2		6.57E5	5.98E3
[illegible]A3		7.26E5	5.60E3
IIIA4		4.24E5	3.28E3
IIIB1		9.44E5	7.58E3
IIIB2		1.50E5	1.71E3
IIIB3		6.65E5	5.05E3
IIIB4		5.68E5	4.94E3
IIIC1		8.91E5	6.35E3
IIIC2		9.94E5	6.92E3
IIIC3	↓	6.35E5	4.76E3

XN	BCECF	2A int	1A int
IA1	↓	2.55E5	3.51E5
IA2		4.63E5	7.34E5
IA3		7.17E5	1.23E6
IA4		8.35E5	1.35E6
IB1		4.99E5	9.76E5
IB2		6.92E5	1.09E6
IB3		3.05E5	5.62E5
IB4		1.53E5	2.50E5
IC1		2.74E5	4.35E5
IC2		7.60E5	1.21E6
IC3		7.34E5	1.01E6
IC4		5.56E5	9.46E5
IIA1		5.70E5	9.54E5
IIA2		1.87E5	2.80E5
IIA3		2.40E5	3.46E5
IIA4		4.74E5	7.34E5
IIB1		7.31E5	1.20E6
IIB2		5.72E5	9.06E5
IIB3	↓	6.38E5	1.06E6

	Dye	2A	1A
#B4	BCECF	6.39E5	9.51E5
#C1		3.10E5	4.93E5
#C2		1.74E5	3.76E5
#C3		3.46E5	6.01E5
#C4		7.14E5	1.13E6
#A1		6.20E5	9.15E5
#A2		5.72E5	1.07E6
#A3		5.58E5	1.02E6
#A4		4.30E5	7.88E5
#B1		1.81E5	2.66E5
#B2		3.82E5	5.93E5
#B3		7.51E5	1.26E6
#B4		5.88E5	8.89E5
#C1		5.41E5	9.25E5
#C2		6.98E5	1.13E6
#C3		3.88E5	7.27E5
IA1	SNAFL	4.35E5	1.77E5
IA2		6.05E5	2.97E5
IA3		4.79E5	2.01E5
IA4		2.03E6	6.57E5
IB1		9.56E5	2.62E5
IB2		1.94E6	6.53E5
IB3		1.77E6	4.75E5
IB4		5.04E5	9.77E4
IC1		1.58E6	4.64E5
IC2		1.16E6	3.49E5
IC3		1.81E6	6.15E5
IC4		1.39E6	4.12E5
IIA1		2.35E6	8.98E5
IIA2		1.76E6	4.37E5
IIA3		9.51E5	1.14E5
IIA4		1.88E6	6.35E5
IIB1		1.93E6	8.35E5
IIB2		5.55E5	9.74E4
IIB3		1.70E6	5.22E5
IIB4		2.34E6	8.81E5
IIC1		1.54E6	3.57E5
IIC2		1.01E6	2.39E5

AN	DYE	2A	1A
IC3	BSNAFL	1.81E6	5.61E5
IC4		2.22E6	9.85E5
IIA		9.53E5	3.11E5
IIA2		1.88E6	5.78E5
IIA3		2.49E6	9.92E5
IIA4		1.83E6	4.77E5
IIB1		1.09E6	2.17E5
IIB2		1.50E6	5.00E5
IIB3		2.30E6	1.06E6
IIB4		3.15E5	6.23E4
IIC1		1.88E6	6.36E5
IIC2		2.40E6	1.01E6
IIC3		1.58E6	4.25E5
IA1	FLUO	9.45E4	6.28E3
IA2		1.64E5	6.68E3
IA3		9.30E4	7.53E3
IA4		6.33E4	7.42E3
IB1		1.03E5	6.34E3
IB2		9.63E4	6.13E3
IB3		1.38E5	6.22E3
IB4		7.24E4	4.84E3
IC1		1.13E5	7.33E3
IC2		1.40E5	8.28E3
IC3		1.23E5	7.37E3
IC4		1.81E5	6.34E3
IIA1		1.15E5	6.55E3
IIA2		1.72E5	6.26E3
IIA3		5.81E4	5.09E3
IIA4		1.46E5	6.28E3
IIB1		1.16E5	7.03E3
IIB2		7.48E4	1.12E4
IIB3		1.66E5	7.46E3
IIB4		1.40E5	6.74E3
IIC1		1.78E5	6.28E3
IIC2		1.06E5	5.02E3
IIC3		1.45E5	5.61E3
IIC4		1.02E5	6.91E3
IIA1		6.11E4	5.79E3

DYE	2A	1A
II A2 FLUO	1.45E5	6.83E3
II A3	1.24E5	6.78E3
II A4	1.69E5	6.59E3
II B1	9.55E4	4.79E3
II B2	1.58E5	6.14E3
II B3	1.38E5	7.25E3
II B4	1.16E5	6.54E3
II C1	1.63E5	6.53E3
II C2	1.20E5	5.85E3
II C3	~~1.27~~E5	6.03E3
	2.21	
I A1 SBFI	1.50E5	8.68E4
I A2	1.43E5	9.24E4
I A3	3.07E5	1.32E5
I A4	2.00E5	9.76E4
I B1	1.86E5	1.04E5
I B2	1.63E5	9.71E4
I B3	1.40E5	8.03E4
I B4	1.79E5	9.35E4
I C1	2.11E5	1.13E5
I C2	4.41E5	1.38E5
I C3	1.51E5	8.18E4
I C4	1.24E5	8.21E4
II A1	1.44E5	9.28E4
II A2	1.27E5	7.83E4
II A3	1.34E5	8.30E4
II A4	1.36E5	9.15E4
II ~~A5~~ B1	2.62E5	1.27E5
II B2	1.42E5	8.18E4
II B3	1.33E5	8.29E4
II B4	1.29E5	8.64E4
II C1	1.16E5	7.40E4
II C2	1.21E5	7.76E4
II C3	1.73E5	1.03E5
II C4	2.01E5	9.46E4
III A1	1.48E5	8.33E4
III A2	1.28E5	8.39E4
III A3	1.33E5	8.66E4
III A4	1.26E5	7.83E4

WI	DYE	2A	1A
IIB1	SBFI	1.22E5	7.58E4
IIB2		1.28E5	7.97E4
IIB3		1.80E5	1.00E5
IIB4		1.49E5	9.84E4
IIC1		1.54E5	8.98E4
IIC2		1.30E5	8.47E4
IIC3	↓	1.40E5	8.02E4
IIA1	PBFI	1.51E5	9.51E4
IIA2		1.56E5	1.05E5
IIA3		2.42E5	1.20E5
IIA4		3.62E5	2.01E5
IIB1		1.58E5	1.17E5
IIB2		2.10E5	1.66E5
IIB3		1.40E5	9.02E4
IIB4		1.36E5	8.76E4
IIC1		1.47E5	9.67E4
IIC2		3.92E5	1.72E5
IIC3		2.88E5	1.19E5
IIC4		1.87E5	1.33E5
IIIA1		1.62E5	1.31E5
IIIA2		1.22E5	8.57E4
IIIA3		1.42E5	9.51E4
IIIA4		1.42E5	9.66E4
IIIB1		2.92E5	1.44E5
IIIB2		1.95E5	8.46E4
IIIB3		1.50E5	9.82E4
IIIB4		2.21E5	1.67E5
IIIC1		1.47E5	8.73E4
IIIC2		9.70E4	6.97E4
IIIC3		1.71E5	1.06E5
IIIC4		5.25E5	1.62E5
IIIA1		2.31E5	1.12E5
IIIA2		1.69E5	1.12E5
IIIA3		1.65E5	1.16E5
IIIA4		1.43E5	8.43E4
IIIB1		1.37E5	9.76E4
IIIB2		1.59E5	1.11E5
IIIB3	↓	2.74E5	1.23E5

1/16/96 cont

	DYE	2A	1A
B4	PBFI	2.46E5	1.58E5
A1	↓	3.49E5	2.59E5
B2		1.81E5	1.31E5
C3		1.42E5	9.34E4

1/18/96

Ran short PT1 experiment with Tomatine (stock 50mg/L – 1/18/96). Saved on DISK 214

SCAN	CONCEN.	Intercept		% Control
1	NC	1.81E5	(1.825 NC)	
2	25mg/L	7.99E5		437.8%
3	50mg/L	7.50E5		410.9%
4	NC	1.84E5		
5	25mg/L	9.41E5		515.6%
6	50mg/L	7.68E5		420.8%

Used nanoject to inject embryos (5) but broke 3 of them. Made up 4% Fluoro-Gold in Fetax. Put in refridgerator + covered w/ foil. Embryos were layed today. Used 23 nl (nL) per "beep". Used dissecting scope + Bausch + Lomb light source.

1/19/96

Ran PTI experiment with α-Chaconine VS. Folic Acid + Tomatine. α-Chac Stock was made 1/9/96 + Tomatine stock was made 1/18/96. Both stock were 50mg/L. Folic Acid stock was made 1/19/96 100mg/L. All pH's were between 6.2-6.8. Albino embryos were used. Scans 1-17 used di-4 from 11-21-95. Scans 18-4 used di-4 from 1/19/96. Disk #14

AN	CONCEN.	ml FA	ml αC	Intercept		% Cont.
1A	NC	0	0	2.08E5		
2A	NC	0	0	2.03E5	1.94E5	100%
3A	NC	0	0	1.7E5		
4A	20mg/L FA, 0 αC	2	0	1.97E5		101.5
5A	30mg/L FA, 0 αC	3	0	2.21E5		113.9
6A	"	3	0	2.29E5		118.0
7A	"	3	0	2.46E5		126.8
8A	40mg/L FA, 0 αC	4	0	1.63E5		84.0
9A	"	4	0	3.04E5		156.7
10A	"	4	0	1.96E5		101.0
11A	20mg/L FA, 2.5 αC	2	.5	2.14E5		110.3
12A	30mg/L FA, 2.5 αC	3	.5	2.00E5		103.1
13A	"	3	.5	1.65E5		85.1
14A	"	3	.5	1.57E5		80.9
15A	40mg/L FA, 2.5 αC	4	.5	1.91E5		98.5
16A	"	4	.5	2.15E5		110.8
17A	"	4	.5	1.69E5		87.1
18A	NC (1/19/96 di-4)	0	0	2.12E5		109.3
19A	20mg/L FA, 5 αC	2	1	1.86E5		95.9
20A	30mg/L FA, 5 αC	3	1	2.53E5		130.4
21A	"	3	1	2.45E5		126.3
22A	"	3	1	2.32E5		119.6
23A	40mg/L FA, 5 αC	4	1	2.11E5		108.8
24A	"	4	1	2.38E5		122.7
25A	"	4	1	2.58E5		132.9
26A	20mg/L FA, 10 αC	2	2	3.10E5		159.8
27A	30mg/L FA, 10 αC	3	2	2.97E5		153.1
28A	"	3	2	2.97E5		153.1
29A	"	3	2	7.96E5		410.3

	CONTENT.	ml FA	ml aC	Intercept	% Control
30A	40mg/L FA, 10aC	4	2	5.77E5	297.4
A	"	4	2	6.78E5	349.5
A	"	4	2	4.32E5	222.7
3A	50mg/L aChac (PC)	0	10	1.07E6	551.5
4A	NC	0	0	2.75E5	use 2.44E5
5A	10 mg/L tomatine	2 ml tomatine		6.85E5	280.7
6A	25 "	5 ml tomatine		8.18E5	335.2
7B	50 "	10 ml tomatine		1.02E6	418.0
8A	NC			2.50E5	~~102.5~~
9A	10 mg/L tomatine	2 ml tomatine		8.45E5	346.3
0A	25 "	5ml "		7.58E5	310.7
1A	50 "	10 ml "		9.32E5	382.0
2A	NC			2.07E5	
3A	10mg/L tomatine	2 ml	Tomatine	9.46E5	387.8
4A	25 mg/L tomatine	5 ml	"	6.31E5	258.6
5A	50mg/L tomatine	10 ml	"	7.70E5	315.6

aChac data + graphs saved on dr3\c 214 as 2AC1_19.GP, 5AC1_19.GP, 10AC1_19.GP, AC1_19.IPG

1/23/96

Set up FETAX experiment using T-BEBC cherry red, T-BEBC cherry green + T-1345-4 green tomatoe. All stocks were 2g/L made 12-21-95. Another stock was made up for the smaller concentrations (200mg/L).

From the 2g/L stock:

2g/L -	10 ml stock	0 ml FTX
1g/L -	5ml stock	5 ml "
0.5g/L -	2.5ml stock	7.5ml "
0.25g/L -	1.25ml stock	8.75ml "

From the 200mg/L stock:

200mg/L -	10ml stock	0ml FTX
100mg/L -	5ml "	5ml "
50mg/L -	2.5ml "	7.5ml "
25mg/L -	1.25ml "	8.75ml "
10mg/L -	0.5ml "	9.5ml "
5mg/L -	0.25ml "	9.75ml "
1mg/L -	0.1 ml "	9.9ml "

1/23/96 Con't

DISH	CONCENT.	DAY0	DAY1	DAY2	DAY3	DAY4
1	NC	25	25	25	25	24
2	2g/L cherry red	25	23	3	2	1
3	1g/L "	25	25	19	16	16
4	0.5g/L "	25	24	22	22	21
5	0.25g/L "	25	25	25	25	~~21~~ 25
6	NC	25	25	25	24	21
7	2g/L cherry green	25	25	23	23	23
8	1g/L "	25	25	24	24	23
9	0.5g/L "	25	25	24	24	23
10	0.25g/L "	25	24	22	22	22
11	NC	25	25	25	25	23
12	2g/L 1345-4 green	25	22	9	5	2
13	1g/L "	25	24	21	21	21
14	0.5g/L "	25	24	22	22	22
15	0.25g/L "	25	24	23	23	22
16	NC	25	25	21	21	19
17	~~200mg/L~~ NC	25	25	19	18	15
18	NC	25	25	24	24	23
19	200mg/L cherry red	25	25	~~22~~ ~~21~~	22	21
20	200mg/L "	25	24	24	22	22
21	200mg/L "	25	24	23	23	22
22	100mg/L "	25	25	23	23	23
23	"	25	25	24	24	23
24	"	25	25	22	22	22
25	50mg/L cherry red	25	25	25	25	25
26	"	25	25	~~23~~ 25	25	23
27	"	25	24	~~24~~ 24	23	21
28	25mg/L cherry red	25	25	25	25	25
29	"	25	25	25	25	25
30	"	25	25	~~22~~ 23	23	22
31	10mg/L Cherry red	25	25	24	24	23
32	"	25	25	24	24	24
33	"	25	25	25	25	22
34	5mg/L cherry red	25	25	24	24	24
35	"	25	25	24	22	22
36	"	25	25	24	24	24
37	1mg/L Cherry red	25	24	24	24	24
38	"	25	25	24	23	22
39	"	25	25	25	23	23

	CONCENT.	DAY0	DAY1	DAY2	DAY3	DAY4
1	200mg/L cherry green	25	25	21	20	20
2	"	25	25	22	22	21
3	"	25	25	18	18	16
4	100mg/L cherry green	25	25	21	21	21
5	"	25	24	21	19	18
6	"	25	25	23	23	23
7	50mg/L cherry green	25	25	24	24	22
8	"	25	25	22	22	22
9	"	25	25	24	23	23
10	25mg/L cherry green	25	25	24	24	23
11	"	25	25	20	20	19
12	"	25	25	23	23	23
13	10mg/L cherry green	25	25	25	25	23
14	"	25	25	24	24	24
15	"	25	25	23	23	20
16	5mg/L cherry green	25	25	25	24	24
17	"	25	25	23	23	23
18	"	25	25	25	25	24
19	1mg/L cherry green	25	25	24	24	23
20	"	25	25	25	25	24
21	"	25	25	24	24	22
22	200mg/L 1345-4 green	25	24	24	24	22
23	"	25	25	25	25	23
24	"	25	25	23	22	20
25	100 mg/L 1345-4 green	25	25	24	24	224
26	"	25	25	24	24	22
27	"	25	25	23	23	21
28	50mg/L 1345-4 green	25	25	24	22	22
29	"	25	25	23	23	23
30	"	25	25	25	24	24
31	25mg/L 1345-4 green	25	25	22	22	22
32	"	25	25	25	25	25
33	"	25	25	25	23	23
34	10mg/L 1345-4 green	25	25	25	25	25
35	"	25	25	23	23	22
36	"	25	25	22	22	21
37	5mg/L 1345-4 green	25	25	25	24	22
38	"	25	24	23	23	22
39	"	25	23	22	22	20

1/23/96 Con't

DISH	CONCENT.	DAY0	DAY1	DAY2	DAY3	DAY4
79	1 mg/L 1345-4 green	25	25	23	22	21
80	"	25	25	23	23	22
81	"	25	25	24	22	21
						↑ 3% Formalin

Spreadsheet saved as TOMTN123.WQ1 (high conc.)
TOM1_23.WQ1 (low conc.)
DISK214

1/25/96
PT 1 experiment with αChac + folic acid using albino embryos. αChac stock is 50mg/L made 1/25/96 + folic acid stock is 100mg/L made 1/25/96. All pH's are between 6.6 - 6.8. DISK216.

SCAN	CONCENT.	ml FA	ml αChac	Intercept	% Control
1	NC	0	0	1.42E5	
2	NC	0	0	1.49E5	1.60E5
3	NC	0	0	1.88E5	
4	PC (50mg/L αC)	0	10	3.83E5	239.4
5	5mg/L FA, 0αC	.5	0	1.32E5	82.5
6	"	.5	0	1.70E5	106.3
7	10mg/L FA, 0αC	1	0	1.50E5	93.8
8	"	1	0	1.63E5	101.9
9	20mg/L FA, 0αC	2	0	1.86E5	116.3
10	"	2	0	1.57E5	98.1
11	30mg/L FA, 0αC	3	0	1.73E5	108.1
12	"	3	0	1.40E5	87.5
13	40mg/L FA, 0αC	4	0	1.74E5	108.8
14	"	4	0	2.09E5	130.6
15	5 FA, 2.5αC	.5	.5	1.74E5	108.8
16	"	.5	.5	1.56E5	97.5
17	10 FA, 2.5αC	1	.5	1.79E5	111.9
18	"	1	.5	1.30E5	81.3
19	20 FA, 2.5αC	2	.5	1.56E5	97.5
20	"	2	.5	1.79E5	111.9
21	30 FA, 2.5αC	3	.5	1.33E5	83.1
22	"	3	.5	1.59E5	99.4
23	40 FA, 2.5αC	4	.5	1.32E5	82.5

N	CONCENT.	ml FA	ml aChac	Intercept	% Control
	40FA, 2.5aC	4	.5	1.60 E5	100%
	5 FA, 5 aC	.5	1	1.65 E5	103.1
	"	.5	1	1.50 E5	93.8
	10FA, 5aC	1	1	1.72 E5	107.5
	"	1	1	1.59 E5	99.4
	20FA, 5aC	2	1	1.66 E5	103.8
	"	2	1	1.53 E5	95.6
	30FA, 5aC	3	1	1.77 E5	110.6
	"	3	1	1.53 E5	95.6
	40FA, 5aC	4	1	1.52 E5	95.0
	"	4	1	1.99 E5	124.4
	5FA, 10 aC	.5	2	1.82 E5	113.8
	"	.5	2	3.32 E5	207.5
	10FA, 10aC	1	2	2.87 E5	179.4
	"	1	2	2.66 E5	166.3
	20FA, 10aC	2	2	2.38 E5	148.8
	"	2	2	3.56 E5	222.5
	30FA, 10aC	3	2	1.53 E5	95.6
	"	3	2	2.15 E5	134.4
	40FA, 10aC	4	2	2.15 E5	134.4
	"	4	2	1.87 E5	116.9
	2.5 mg/L aC	0	.5	1.61 E5	100.6
	5 mg/L aC	0	1	1.61 E5	100.6
	10 mg/L aC	0	2	6.06 E5	378.8

Check dISK 216 For saved data in "inplot"
2 AC1-25.GP, 5 AC1-25.GP, 10 AC1-25.GP, AC1-25.IPG. Averaged data → saved as AVG2AC-GP, AVG5AC-GP, AVG10AC-GP, AVAC1-25.IPG. DISK-216,

1/26/96
Ran Chicken experiment. DISK 217. DAY30.
Spreadsheet data - DISK220, Page 88 - modifications

Di-4

SCAN	2A DYE	1A	2A BCECF	1A	2A FLUO	1A
IA1	6.51E5	-1.13E4 ✓	2.75E5	2.10E5 ✓	2.25E5	1.40E3 ✓
IA2	~~-1.13E4~~ 8.33E5	-1.11E4 ✓	9.78E5	1.16E6 ✓	5.17E5	1.31E4 ✓
IA3	~~8.33E5~~ 1.94E6	294.57 ✓	1.12E6	1.06E6 ✓	2.67E5	2.20E3 ✓
IA4	~~-1.11E4~~ 1.17E6	-5.61E3 ✓	1.43E6	1.57E6 ✓	1.01E5	3.16E3 ✓
IB1	9.64E5	-1.02E4 ✓	7.28E5	6.47E5 ✓	3.93E5	5.78E3 ✓

SCAN	Di-4 DYE 2A	1A	BCECF 2A	1A	FLUO 2A	1A
IB3	7.04E5	-9.32E3 ✓	7.22E5	7.22E5 ✓	3.22E5	2.17E3 ✓
IB4	1.11E6	-8.96E3 ✓	3.06E5	2.81E5 ✓	2.27E5	938.07 ✓
IC1	5.38E5	-1.23E4 ✓	8.66E5	8.00E5 ✓	3.26E5	1.45E3 ✓
IC2	1.18E6	-6.00E3 ✓	4.90E5	5.57E5 ✓	4.15E5	3.24E3 ✓
IC3	1.06E6	-8.84E3 ✓	9.13E5	9.66E5 ✓	8.92E4	866.18 ✓
IC4	1.09E6	-8.04E3 ✓	6.88E5	5.75E5 ✓	2.33E5	1.66E3 ✓
IIA1	6.01E5	-1.29E4 ✓	7.82E5	7.06E5 ✓	3.66E5	2.00E3 ✓
IIA2	1.14E6	-8.75E3 ✓	6.55E5	5.72E5 ✓	5.56E5	5.03E3 ✓
IIA3	1.64E6	-2.80E3 ✓	4.33E5	3.79E5 ✓	2.57E5	1.72E3 ✓
IIA4	1.32E6	-6.55E3 ✓	1.04E6	9.91E5 ✓	6.37E5	5.42E3 ✓
IIB1	1.16E6	-6.72E3 ✓	1.56E6	1.71E6 ✓	1.22E5	3.60E3 ✓
IIB2	1.35E6	-6.43E3 ✓	1.55E6	1.79E6 ✓	9.28E4	3.45E3 ✓
IIB3	1.67E6	-2.64E3 ✓	1.38E6	1.45E6 ✓	4.41E5	5.39E3 ✓
IIB4	8.48E5	-1.05E4 ✓	1.42E6	1.43E6 ✓	2.31E5	3.03E3 ✓
IIC1	1.48E6	-5.03E3 ✓	6.41E5	4.82E5 ✓	4.24E5	3.84E3 ✓
IIC2	8.80E5	-1.03E4 ✓	5.48E5	5.19E5 ✓	1.53E5	1.94E3 ✓
IIC3	9.72E5	-8.99E3 ✓	8.50E5	9.24E5 ✓	7.36E5	6.05E3 ✓
IIC4	1.01E6	-8.01E3 ✓	8.31E5	8.59E5 ✓	1.57E5	3.39E3 ✓
IIIA1	1.52E6	-4.03E3 ✓	1.38E6	1.45E6 ✓	1.03E5	3.94E3 ✓
IIIA2	9.57E5	-6.16E3 ✓	6.38E5	6.10E5 ✓	2.36E5	2.99E3 ✓
IIIA3	1.49E6	-2.62E3 ✓	6.21E5	5.36E5 ✓	2.97E5	2.97E3 ✓
IIIA4	1.18E6	-4.04E3 ✓	4.01E5	3.61E5 ✓	4.48E5	4.67E3 ✓
IIIB1	1.10E6	-2.44E3 ✓	1.90E5	1.84E5 ✓	2.84E5	2.94E3 ✓
IIIB2	1.09E6	-2.67E3 ✓	5.66E5	5.94E5 ✓	4.99E5	4.00E3 ✓
IIIB3	1.68E6	5.75E3 ✓	7.18E5	7.85E5 ✓	2.77E5	3.86E3 ✓
IIIB4	1.29E6	1.25E3 ✓	8.18E5	8.63E5 ✓	1.42E5	2.73E3 ✓
IIIC1	2.38E6	1.37E4 ✓	7.23E5	6.66E5 ✓	4.54E5	4.42E3 ✓
IIIC2	1.74E6	4.88E3 ✓	9.27E5	8.39E5 ✓	2.91E5	3.09E3 ✓
IIIC3	1.40E6	2.14E3 ✓	4.96E5	4.48E5 ✓	5.90E5	3.95E3 ✓
IVA1	1.09E6	-7.12E3 ✓	2.09E5	1.95E5 ✓	2.09E5	1.83E3 ✓
IVA2	1.46E6	-2.36E3 ✓	5.94E5	6.86E5 ✓	5.22E5	4.69E3 ✓
IVA3	2.18E6	6.17E3 ✓	6.01E5	6.20E5 ✓	2.55E5	4.96E3 ✓
IVA4	1.62E6	-988.89 ✓	9.61E5	1.00E6 ✓	1.09E5	3.51E3 ✓
IVB1	1.29E6	-4.03E3 ✓	9.99E5	9.82E5 ✓	2.78E5	2.04E3 ✓
IVB2	1.21E6	-4.47E3 ✓	6.69E5	6.88E5 ✓	3.12E5	3.03E3 ✓
IVB3	1.07E6	-5.91E3 ✓	4.96E5	3.95E5 ✓	5.27E5	4.62E3 ✓
IVB4	9.80E5	-7.40E3 ✓	4.52E5	4.10E5 ✓	458E5	2.88E3 ✓
IVC1	1.07E6	-4.03E3 ✓	2.57E5	3.83E5 ✓	5.17E5	4.23E3 ✓
IVC2	1.43E6	-1.64E3 ✓	8.19E5	8.08E5 ✓	3.44E5	4.65E3 ✓

Di-4

SCAN	2A DYE	1A	2A BCECF	1A	2A FLUO	1A
IIC3	1.42E6	-2.48E3 ✓	1.12E6	1.19E6 ✓	9.93E4	3.17E3 ✓
IIC4	8.05E5	-7.14E3 ✓	6.94E5	6.39E5 ✓	3.76E5	3.72E3 ✓
IIA1	9.55E5	-9.48E3 ✓	7.27E5	6.40E5 ✓	1.85E5	3.85E3 ✓
IIA2	1.14E6	-8.03E3 ✓	7.37E5	7.20E5 ✓	4.74E5	5.02E3 ✓

SCAN	2A SBFI	1A	2A PBFI	1A
IA1	7.74E4	7.60E4 ✓	1.16E5	1.68E5 ✓
IA2	3.35E5	2.11E5 ✓	1.55E5	2.09E5 ✓
IA3	5.46E5	2.11E5 ✓	6.69E5	5.44E5 ✓
IA4	1.46E5	6.22E4 ✓	2.13E5	9.86E4 ✓
IB1	1.67E5	8.51E4 ✓	1.85E5	1.40E5 ✓
IB2	2.02E5	1.63E5 ✓	1.85E5	2.69E5 ✓
IB3	1.16E5	3.67E4 ✓	1.71E5	1.90E5 ✓
IB4	9.89E4	6.06E4 ✓	1.09E5	1.45E5 ✓
IC1	1.81E5	1.60E5 ✓	2.13E5	2.71E5 ✓
IC2	4.49E5	3.30E5 ✓	1.16E6	6.25E5 ✓
IC3	1.65E5	6.36E4 ✓	2.31E5	1.59E5 ✓
IC4	1.67E5	1.48E5 ✓	1.94E5	3.18E5 ✓
IIA2	8.61E4	6.13E4 ✓	1.47E5	2.65E5 ✓
IIA2	7.11E4	3.22E4 ✓	1.64E5	1.84E5 ✓
IIA3	5.02E4	2.28E4 ✓	9.56E4	1.45E5 ✓
IIA4	1.70E5	1.25E5 ✓	2.45E5	4.61E5 ✓
IIB1	3.30E5	2.28E5 ✓	3.89E5	3.96E5 ✓
IIB2	1.75E5	7.57E4 ✓	1.90E5	9.38E4 ✓
IIB3	1.57E5	235.63 ✓	2.07E5	3.58E5 ✓
IIB4	1.97E5	9.07E4 ✓	1.56E5	1.85E5 ✓
IIC1	1.60E5	6.39E4 ✓	1.03E5	1.29E5 ✓
IIC2	7.77E4	7.44E4 ✓	1.37E5	2.16E5 ✓
IIC3	3.22E5	2.75E5 ✓	1.65E5	2.18E5 ✓
IIC4	3.14E5	1.61E5 ✓	4.07E5	3.25E5 ✓
IIIA1	1.89E5	8.38E4 ✓	1.78E5	1.12E5 ✓
IIIA2	1.18E5	5.62E4 ✓	1.78E5	3.10E5 ✓
IIIA3	1.62E5	1.78E5 ✓	1.82E5	2.53E5 ✓
IIIA4	1.64E5	1.17E5 ✓	1.89E5	2.57E5 ✓
IIIB1	9.69E4	7.62E4 ✓	1.03E5	1.51E5 ✓
IIIB2	1.20E5	9.26E4 ✓	1.66E5	2.84E5 ✓
IIIB3	2.46E5	1.66E5 ✓	4.68E5	3.47E5 ✓
IIIB4	1.71E5	7.07E4 ✓	1.74E5	1.12E5 ✓
IIIC1	1.53E5	1.01E5 ✓	1.94E5	3.02E5 ✓

	2A SPEI	1A	2A PBFI	1A
IIC2	1.31E5	1.13E5 ✓	1.86E5	4.05E5 ✓
IIC3	8.16E4	3.48E4 ✓	2.13E5	2.57E5 ✓
IA1	1.21E5	1.04E5 ✓	8.48E4	1.26E5 ✓
IA2	1.82E5	1.42E5 ✓	2.62E5	3.28E5 ✓
IA3	3.43E5	1.77E5 ✓	4.70E5	5.49E5 ✓
IA4	2.40E5	8.60E4 ✓	3.37E5	2.41E5 ✓
IIB1	1.21E5	1.25E5 ✓	1.66E5	2.18E5 ✓
IIB2	1.71E5	1.44E5 ✓	1.93E5	3.26E5 ✓
IIB3	1.38E5	6.83E4 ✓	1.87E5	2.59E5 ✓
IIB4	8.67E4	8.45E4 ✓	2.10E5	2.51E5 ✓
IIC1	2.47E5	2.79E5 ✓	1.55E5	2.46E5 ✓
IIC2	2.67E5	1.51E5 ✓	5.34E5	3.48E5 ✓
IIC3	2.37E5	9.26E4 ✓	2.82E5	1.21E5 ✓
IIC4	2.32E5	2.02E5 ✓	2.34E5	4.17E5 ✓
IIA1	1.94E5	1.31E5 ✓	1.19E5	2.43E5 ✓
IIA2	1.55E5	9.53E4 ✓	1.66E5	1.88E5 ✓

1/30/96
Setup FETAX test with high Folic Acid Concentrations + 10mg/L αChac. α-Chac stock (50mg/L) was made 1/30/96 + folic acid stock (100mg/L) was made 1/30/96. PH's were from 6.0 - 6.7.

DISH	Concent.	DAY0	DAY1	DAY2	DAY3	DAY4
1	NC	25				
2	20mg/L FA, 10mg/L αC	25	0			
3	30 FA, 10 αC	25	0			
4	40 FA, 10 αC	25	0	test aborted - embryos not healthy		
5	50 FA, 10 αC	25	0			
6	NC	25				
7	20 FA, 10αC	25	0			
8	30 FA, 10αC	25	0			
9	40 FA, 10αC	25	0			
10	50 FA, 10αC	25	0			

2/6/96

Ran chicken experiment. DISK 218. Day 40.
Spreadsheet data - D13\220. Page 88 has modifications

	1A Di-4	2A	1A BCECF	2A	1A SNAFL	2A
A1	5.32E3	7.88E5 ✓	2.33E5	5.22E5 ✓	7.59E5	6.70E5 ✓
A2	1.23E4	1.67E6 ✓	4.73E5	9.52E5 ✓	7.90E5	7.48E5 ✓
A3	1.35E4	1.78E6 ✓	3.35E5	6.71E5 ✓	1.22E6	1.08E6 ✓
A4	1.23E4	1.70E6 ✓	7.17E5	1.25E6 ✓	7.92E5	6.39E5 ✓
B1	1.03E4	1.49E6 ✓	4.99E5	1.12E6 ✓	1.07E6	1.07E6 ✓
B2	1.41E4	1.84E6 ✓	5.86E5	1.12E6 ✓	8.44E5	8.72E5 ✓
B3	1.98E4	2.36E6 ✓	5.01E5	9.66E5 ✓	8.55E5	8.01E5 ✓
B4	5.97E3	1.10E6 ✓	9.77E4	2.07E5 ✓	5.14E5	5.04E5 ✓
C1	1.14E4	1.66E6 ✓	5.15E5	9.66E5 ✓	1.39E6	1.18E6 ✓
C2	1.61E4	2.03E6 ✓	6.62E5	1.23E6 ✓	1.81E6	1.64E6 ✓
C3	1.96E4	2.27E6 ✓	7.91E5	1.38E6 ✓	8.13E5	6.77E5 ✓
C4	9.24E3	1.44E6 ✓	7.67E5	1.43E6 ✓	6.85E5	6.93E5 ✓
A1	1.66E4	2.04E6 ✓	5.16E5	9.71E5 ✓	1.33E6	1.15E6 ✓
II A2	1.21E4	1.61E6 ✓	5.69E5	1.09E6 ✓	6.43E5	7.15E5 ✓
I A3	1.64E4	2.02E6 ✓	2.34E5	4.95E5 ✓	2.77E5	2.61E5 ✓
I A4	8.16E3	1.10E6 ✓	1.89E5	3.74E5 ✓	8.14E5	7.58E5 ✓
B1	1.52E4	1.77E6 ✓	6.65E5	1.18E6 ✓	1.37E6	1.17E6 ✓
I B2	1.25E4	1.63E6 ✓	7.49E5	1.23E6 ✓	9.28E5	7.50E5 ✓
I B3	1.17E4	1.53E6 ✓	5.59E5	1.18E6 ✓	8.66E5	9.24E5 ✓
I B4	1.16E4	1.57E6 ✓	6.61E5	1.24E6 ✓	1.23E6	1.13E6 ✓
C1	9.85E3	1.32E6 ✓	4.81E5	9.87E5 ✓	7.99E5	8.76E5 ✓
C2	9.81E3	1.45E6 ✓	2.53E5	5.62E5 ✓	4.24E5	4.56E5 ✓
C3	5.34E3	7.62E5 ✓	6.48E5	1.10E6 ✓	7.23E5	6.71E5 ✓
C4	2.60E4	2.64E6 ✓	7.68E5	1.37E6 ✓	1.31E6	1.20E6 ✓
A1	1.14E4	1.59E6 ✓	6.51E5	1.12E6 ✓	7.44E5	6.39E5 ✓
A2	1.19E4	1.56E6 ✓	5.10E5	1.14E6 ✓	7.57E5	7.20E5 ✓
A3	9.46E3	1.38E6 ✓	7.25E5	1.34E6 ✓	7.92E5	7.73E5 ✓
A4	3.64E4	3.21E6 ✓	2.98E5	7.03E5 ✓	5.34E5	6.33E5 ✓
B1	9.45E3	1.43E6 ✓	1.81E5	4.21E5 ✓	5.08E5	5.19E5 ✓
B2	6.90E3	1.01E6 ✓	2.83E5	5.57E5 ✓	8.64E5	7.92E5 ✓
II B3	3.08E4	2.91E6 ✓	6.00E5	1.15E6 ✓	1.69E6	1.46E6 ✓
B4	1.18E4	1.66E6 ✓	7.98E5	1.33E6 ✓	9.22E5	7.86E5 ✓
C1	5.35E3	8.74E5 ✓	6.02E5	1.27E6 ✓	1.44E6	1.43E6 ✓
C2	2.68E4	2.78E6 ✓	5.52E5	1.14E6 ✓	9.21E5	9.27E5 ✓
C3	1.32E4	1.86E6 ✓	4.36E5	1.06E6 ✓	4.93E5	5.81E5 ✓

SAN	1A Di-4	2A	1A BCECE	2A	1A SNAFL	2A
IIA1	5.16E3	8.69E5 ✓	1.15E5	2.40E5 ✓	3.61E5	3.88E5 ✓
IIA2	6.79E3	1.12E6 ✓	5.74E5	8.64E5 ✓	7.69E5	6.36E5 ✓
IIA3	4.58E4	3.68E6 ✓	8.06E5	1.34E6 ✓	1.42E6	1.27E6 ✓
IIA4	1.03E4	1.52E6 ✓	9.04E5	1.54E6 ✓	1.41E6	1.28E6 ✓
IIB1	5.67E3	9.12E5 ✓	5.07E5	1.07E6 ✓	9.49E5	9.10E5 ✓
IIB2	9.39E3	1.48E6 ✓	6.66E5	1.36E6 ✓	7.96E5	8.33E5 ✓
IIB3	2.07E4	2.35E6 ✓	5.28E5	1.16E6 ✓	9.99E5	9.77E5 ✓
IIB4	9.84E3	1.59E6 ✓	1.25E5	2.27E5 ✓	3.89E5	4.26E5 ✓
IIC1	5.67E3	9.67E5 ✓	1.73E5	3.76E5 ✓	1.34E6	1.16E6 ✓
IIC2	1.34E4	1.81E6 ✓	8.43 ~~8.48~~E5	1.54E6 ✓	1.32E6	1.18E6 ✓
IIC3	1.07E4	1.54E6 ✓	8.66E5	1.48E6 ✓	9.23E5	7.75E5 ✓
IIC4	9.45E3	1.51E6 ✓	7.02E5	1.40E6 ✓	7.10E5	7.80E5 ✓
IIIA1	7.31E3	1.01E6 ✓	6.08E5	1.23E6 ✓	8.89E5	9.30E5 ✓
IIIA2	7.86E3	1.26E6 ✓	5.77E5	1.25E6 ✓	5.39E5	6.51E5 ✓

SAN	1A Fluo-3	2A	1A SBFI	2A	1A PBFI	2A
IA1	6.32E4 ✓		3.61E5	4.34E5 ✓	1.55E5	1.65E5 ✓
IA2	1.30E5 ✓		2.71E5	3.27E5 ✓	1.91E5	2.06E5 ✓
IA3	3.06E4 ✓		3.40E5	4.89E5 ✓	2.26E5	3.63E5 ✓
IA4	2.71E4 ✓		2.27E5	3.82E5 ✓	1.32E5	1.62E5 ✓
IB1	1.19E5 ✓		2.56E5	3.66E5 ✓	1.52E5	1.48E5 ✓
IB2	7.38E4 ✓		3.13E5	3.52E5 ✓	1.55E5	1.48E5 ✓
IB3	1.51E5 ✓	1.11E5 →	1.96E5 IB3	2.86E5 ✓	7.02E4	8.87E4 ✓
IB4	~~1.11E5~~	1.42 ~~1.11~~E5 ✓	2.31E5 IB4	2.66E5 ✓	8.10E4	IB4 9.63E4 ✓
IC1			2.07E5 IC1	2.54E5 ✓	3.16E5	IC1 3.02E5 ✓
IC2		4.39E4 ~~1.42E5~~ ✓	3.37E5	4.90E5 ✓	1.56E5 ✓	IC2 3.30E5 ✓
IC3		1.66 ~~4.39~~E4 ✓	2.08E5	3.20E5 ✓	1.05E5 ✓	IC3 1.60E5 ✓
IC4			2.94E5	3.73E5 ✓	1.53E5 ✓	IC4 1.53E5 ✓
IIA1	7.27E4 ✓		2.64E5	2.97E5 ✓	1.87E5	~~2.66E5~~ 1.78E5 ✓
IIA2	1.55E5 ✓		1.98E5	2.94E5 ✓	1.01E5	1.19E5 ✓
IIA3	6.52E4 ✓		1.86E5	2.33E5 ✓	3.76E4	4.85E4 ✓
IIA4	1.42E5 ✓		1.94E5	2.12E5 ✓	7.23E4	8.29E4 ✓
IIB1	3.25E4 ✓		4.67E5	1.03E6 ✓	1.67E5	4.35E5 ✓
IIB2	1.65E4 ✓		2.10E5	3.31E5 ✓	4.50E4	7.79E4 ✓
IIB3	7.06E4 ✓		2.39E5	3.00E5 ✓	8.30E4	1.16E5 ✓
IIB4	7.61E4 ✓		4.24E5	4.18E5 ✓	7.64E4	8.79E4 ✓
IIC1	1.78E5 ✓		2.13E5	2.79E5 ✓	9.31E4	1.11E5 ✓
IIC2	4.14E4 ✓		1.96E5	2.34E5 ✓	7.09E4	8.45E4 ✓
IIC3	1.28E5 ✓		7.40E5	6.17E5 ✓	2.01E4	1.23E5 ✓

XAN	1A FLUO-3 2A	1A SBFI 2A		1A PBFI 2A	
IIC4	4.10E4 ✓	4.06E5	6.12E5 ✓	1.63E5	3.33E5 ✓
IIIA1	2.08E4 ✓	1.76E5	2.83E5 ✓	8.45E4	1.77E5 ✓
IIIA2	6.28E4 ✓	2.10E5	2.81E5 ✓	7.78E4	1.14E5 ✓
IIIA3	1.14E5 ✓	2.56E5	2.66E5 ✓	1.38E5	1.33E5 ✓
IIIA4	1.56E5 ✓	2.31E5	3.15E5 ✓	1.05E5	1.20E5 ✓
IIIB1	4.19E4 ✓	1.41E5	1.66E5 ✓	4.76E4	5.83E4 ✓
IIIB2	9.94E4 ✓	1.75E5	2.18E5 ✓	8.61E4	1.07E5 ✓
IIIB3	3.74E4 ✓	3.25E5	4.51E5 ✓	1.18E5	1.87E5 ✓
IIIB4	2.05E4 ✓	2.31E5	3.57E5 ✓	6.62E4	1.22E5 ✓
IIIC1	9.83E4 ✓	3.73E5	4.27E5 ✓	7.39E4	9.90E4 ✓
IIIC2	1.26E5 ✓	2.56E5	3.19E5 ✓	1.54E5	1.50E5 ✓
IIIC3	1.93E5 ✓	2.68E5	3.27E5 ✓	9.05E4	1.05E5 ✓
IVA1	4.62E4 ✓	2.79E5	3.29E5 ✓	4.48E4	5.28E4 ✓
IVA2	1.12E5 ✓	2.96E5	3.55E5 ✓	7.45E4	8.76E4 ✓
IVA3	3.62E4 ✓	3.10E5	4.56E5 ✓	1.45E5	2.17E5 ✓
IVA4	4.61E4 ✓	3.92E5	5.06E5 ✓	6.26E4	1.11E5 ✓
IVB1	1.91E5 ✓	2.85E5	3.04E5 ✓	9.79E4	1.24E5 ✓
IVB2	5.40E4 ✓	2.27E5	3.05E5 ✓	1.34E5	1.29E5 ✓
IVB3	1.83E5 ✓	1.78E5	2.60E5 ✓	7.97E4	1.06E5 ✓
IVB4	8.00E4 ✓	1.77E5	2.06E5 ✓	5.09E4	6.40E4 ✓
IVC1	1.61E5 ✓	6.11E5	5.36E5 ✓	1.25E5	1.42E5 ✓
IVC2	5.31E4 ✓	3.65E5	5.73E5 ✓	1.72E5	4.94E5 ✓
IVC3	3.14E4 ✓	1.78E5	2.65E5 ✓	8.93E4	1.40E5 ✓
IVC4	1.42E5 ✓	1.88E5	2.42E5 ✓	5.43E4	8.99E4 ✓
VA1	1.03E5 ✓	2.80E5	3.13E5 ✓	1.34E5	1.43E5 ✓
VA2	9.42E4 ✓	7.67E4	1.05E5 ✓	8.13E4	9.76E4 ✓

2/8/96

FETAX experiment with T-BEBC cherry red, T-BEBC cherry green and T-1345-4 green tomatoe. All stocks were 2g/L made 2/8/96. Then 200mg/L stocks were made from the 2g/L stocks. The concentrations were made as follows:

From 2g/L stock:

	ml stock	ml FETAX
2g/L =>	10	0
1g/L =>	5	5
0.5g/L =>	2.5	7.5
0.25g/L =>	1.25	8.75

From 200mg/L stock:

	ml stock	ml FETAX
200mg/L =>	10	0
100mg/L =>	5	5
50mg/L =>	2.5	7.5
25mg/L =>	1.25	8.75
10mg/L =>	0.5	9.5
5mg/L =>	0.25	9.75
1mg/L =>	0.1	9.9

Digitized on DISK 225 saved as RED2-8.PRN GREEN2-8.PRN 1345G2-5.PRN

DISH	CONCENTRATION	DAY0	DAY1	DAY2	DAY3	DAY4
1	NC	25	25	25	25	25
2	NC	25	25	25	25	25
3	NC	25	25	25	25	25
4	200mg/L cherry red	25	25	24	24	23
5	"	25	25	24	24	23
6	"	25	25	22	22	21
7	100mg/L cherry red	25	25	25	25	24
8	"	25	25	25	25	24
9	"	25	25	23	23	23
10	50mg/L cherry red	25	25	25	25	25
11	"	25	25	23	23	22
12	"	25	25	25	25	25
13	25mg/L cherry red	25	25	24	24	23
14	"	25	25	24	24	24
15	"	25	25	25	25	25
16	10mg/L cherry red	25	25	24	24	24
17	"	25	25	24	23	23
18	"	25	25	25	23	25

DISH #	CONCEN.	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
19	5mg/L cherry red	25	25	25	25	23
20	"	25	25	25	25	25
21	"	25	25	25	24	23
22	1mg/L cherry red	25	25	25	25	25
23	"	25	25	25	25	25
24	"	25	25	25	26	25
25	200mg/L cherry green	25	24	2	2	2
26	"	25	25	1	1	1
27	"	25	25	2	2	2
28	100mg/L cherry green	25	25	23	21	21
29	"	25	25	24	24	24
30	"	25	25	23	21	21
31	50mg/L cherry green	25	25	25	25	25
32	"	25	25	24	21	21
33	"	25	25	24	23	22
34	25mg/L cherry green	25	25	24	24	24
35	"	25	25	23	21	21
36	"	25	25	24	21	20
37	10mg/L cherry green	25	25	25	25	~~24~~ 25
38	"	25	25	24	24	24
39	"	25	25	26	26	2
40	5mg/L cherry green	25	25	23	23	23
41	"	25	25	25	24	24
42	"	25	25	25	24	24
43	1mg/L cherry green	25	25	25	25	25
44	"	25	25	26	25	25
45	"	25	25	24	24	24
46	200mg/L 1345-4 green	25	25	24	24	24
47	"	25	25	25	25	25
48	"	25	25	25	25	25
49	100mg/L 1345-4 gr.	25	25	24	24	23
50	"	25	25	25	25	24
51	"	25	25	24	24	23
52	50mg/L 1345-4 gr.	25	25	24	23	23
53	"	25	25	25	24	24
54	"	25	25	25	23	23
55	25mg/L 1345-4 gr.	25	25	25	23	22
56	"	25	25	23	22	22
57	"	25	25	25	22	22

FISH	CONCENT.	DAY0	DAY1	DAY2	DAY3	DAY4
58	10mg/L 1345-4 green	25	25	24	~~23~~ 24	24
59	"	25	25	25	~~22~~ ~~23~~	22
60	"	25	25	23	~~22~~ 23	23
61	5 mg/L 1345-4 green	25	25	25	25	25
62	"	25	25	25	25	25
63	"	25	25	24	24	24
64	1 mg/L 1345-4 green	25	25	23	23	23
65	"	25	25	25	25	25
66	"	25	25	25	25	25
67	NC	25	25	24	~~24~~	23
68	NC	25	25	24	24	24
69	NC	25	25	24	~~23~~	20
70	2g/L cherry red	25	25	0	0	0
71	"	25	25	0	0	0
72	"	25	25	0	0	0
73	1g/L cherry red	25	25	0	0	0
74	"	25	25	0	0	0
75	"	25	25	0	0	0
76	0.5g/L cherry red	25	25	~~0~~	0	0
77	"	25	25	10	9	9
78	"	25	25	1	1	1
79	0.25g/L cherry red	25	25	14	12	12
80	"	25	25	17	16	16
81	"	25	25	16	15 (17 dy)	15
82	NC	25	25	25	23	23
83	NC	25	25	25	25	25
84	NC	25	25	25	25	24
85	2g/L cherry green	25	0	0	0	0
86	"	25	0	0	0	0
87	"	25	0	0	0	0
88	1g/L cherry green	25	0	0	0	0
89	"	25	0	0	0	0
90	"	25	0	0	0	0
91	.5g/L cherry green	25	0	0	0	0
92	"	25	2	0	0	0
93	"	25	0	0	0	0
94	.25g/L cherry green	25	19	0	0	0
95	"	25	20	0	0	0
96	"	25	25	0	0	0

#'s	CONCENT.	DAY0	DAY1	DAY2	DAY3	DAY4
97	NC	25	25	21	21	21
98	NC	25	25	25	25	25
99	NC	25	25	25	25	24
100	2g/L 1345-4 green	25	25	0	0	0
101	"	25	25	0	0	0
102	"	25	23	0	0	0
103	1g/L 1345-4 green	25	25	0	0	0
104	"	25	25	0	0	0
105	"	25	25	1	1	1
106	.5g/L 1345-4 green	25	25	16	15	15
107	"	25	25	18	17	17
108	"	25	25	11	10	10
109	.25g/L 1345-4 green	25	25	23	22	21
110	"	25	25	22	22	22
111	"	25	25	22	21	21

↑ 3% Formalin

Done with albinos.

Saved as TOMTN2_8.WQ1 DISK214

2/13/96

Set up FETAX test with αChac VS. Folic Acid with albinos. αChac stock was made (50mg/L) today from Mendel's αC from 2-9-96. Folic Acid stock was made today, also (100mg/L). pH's were between 6.0 & 6.5.

10mg/L Folic Acid	=>	1 ml	Folic acid	stock
20mg/L "	=>	2 ml	"	"
30mg/L "	=>	3 ml	"	"
40mg/L "	=>	4 ml	"	"
50mg/L "	=>	5 ml	"	"
2.5mg/L αChac	=>	.5 ml	αChac	stock
5 mg/L αChac	=>	1 ml	αChac	stock
10mg/L αChac	=>	2 ml	αChac	stock

#	CONCEN.	DAY0	DAY1	DAY2	DAY3	DAY4
1	NC	25	25	24	24	24
2	NC	25	25	24	24	24
3	10mg/L FA, OAC	25	25	24	24	24
4	"	25	25	25	25	25
5	20mg/L FA, OAC	25	25	24	24	24
6	"	25	25	25	23	23
7	30mg/L FA, OAC	25	25	25	25	25
8	"	25	25	25	25	25
9	40mg/L FA, OAC	25	25	23	23	23
10	"	25	25	25	25	24
11	50mg/L FA, OAC	25	25	24	24	23
12	"	25	25	25	25	25
13	OFA, 2.5mg/L AC	25	25	16	8	7
14	"	25	25	14	14	1
15	10mg/L FA, 2.5mg/L AC	25	25	22	20	18
16	"	25	25	23	16	15
17	20mg/L FA, 2.5mg/L AC	25	25	22	18	17
18	"	25	25	22	~~20~~ 21	21
19	"	25	~~24~~ 25	20	20	18
20	30mg/L FA, 2.5mg/L AC	25	25	25	25	25
21	"	25	25	22	17	16
22	"	25	25	24	24	24
23	40mg/L FA, 2.5mg/L AC	25	25	23	21	17
24	"	25	25	25	24	19
25	"	25	25	25	25	23
26	50mg/L FA, 2.5mg/L AC	25	25	24	24	24
27	"	25	25	22	19	18
28	OFA, 5mg/L AC	25	16	0	0	0
29	"	25	21	0	0	0
30	10mg/L FA, 5mg/L AC	25	25	1	0	0
31	"	25	24	2	0	0
32	20mg/L FA, 5mg/L AC	25	25	1	0	0
33	"	25	25	3	0	0
34	"	25	19	3	0	0
35	30mg/L FA, 5mg/L AC	25	25	3	1	0
36	"	25	24	1	0	0
37	"	25	25	1	0	0
38	40mg/L FA, 5mg/L AC	25	25	2	0	0
39	"	25	25	13	~~16~~	0

DISH	CONCEN	DAY0	DAY1	DAY2	DAY3	DAY4
40	40mg/L FA, 5mg/L AC	25	25	4	4	0
41	50mg/L FA, 5mg/L AC	25	25	3	2	0
42	"	25	25	3	2	0
43	OAC FA, 10mg/L AC	25	0	0	0	0
44	"	25	0	0	0	0
45	10mg/L FA, 10mg/L AC	25	0	0	0	0
46	"	25	0	0	0	0
47	20mg/L FA, 10mg/L AC	25	0	0	0	0
48	"	25	0	0	0	0
49	"	25	0	0	0	0
50	30mg/L FA, 10mg/L AC	25	0	0	0	0
51	"	25	0	0	0	0
52	"	25	0	0	0	0
53	40mg/L FA, 10mg/L AC	25	0	0	0	0
54	"	25	0	0	0	0
55	"	25	0	0	0	0
56	50mg/L FA, 10mg/L AC	25	0	0	0	0
57	"	25	0	0	0	0

↑ 3% Formalin

Saved spreadsheet on D13K214 as D1AC2_13.WQ1, D2AC2_13.WQ1, D3AC2_13.WQ1, D4AC2_13.WQ1.
Digitized on D3K225 saved as ACFA2_13.PRN

2/15/96
Set up TMT concentrations with albino embryos. TMT stock was 10mg/L made 2/14/96.

[TMT]	ml TMT stock	ml FTX	
NC	0	10	set up @
0.1 mg/L	.1	9.9	1:00 pm.
0.25 mg/L	.25	9.75	
0.5 mg/L	.5	9.5	15 embryos per
1 mg/L	1	9	dish.

2/16/96

From 2/15/96 - put 40 ul di-4 into each dish of 10 ml of TMT concentrations. Embryos are 24 hr 4 old. Used 1600 asp. film, 36 ex. Stages 26, 27 (approx.). Fluorescence. Embryos incubated for 27 hours in TMT + 2.5 hrs. in di-4.

Pictures	[TMT]	obj.	Exp. time	
1	NC	10X	1 sec	
2	NC	10X	1 & 2 sec	- didn't turn out
3	NC	10X	2 sec	
4	NC	5X	1 sec	
5	NC	5X	2 sec	
6	.1 mg/L	5X	1 sec	
7	.1 mg/L	5X	2 sec	
8	.1 mg/L	10X	1 sec	
9	.1 mg/L	10X	2 sec	
10	.25 mg/L	10X	1 sec	
11	.25 mg/L	10X	1 sec	- didn't turn out
12	.25 mg/L	10X	2 sec	
13	.25 mg/L	5X	1 sec	
14	.25 mg/L	5X	2 sec	
15	.5 mg/L	5X	1 sec	
16	.5 mg/L	5X	2 sec	
17	.5 mg/L	10X	1 sec	
18	.5 mg/L	10X	2 sec	
19	1 mg/L	10X	1 sec	
20	1 mg/L	10X	2 sec	
21	1 mg/L	5X	1 sec	
22	1 mg/L	5X	2 sec	
23	NC	5X	1 sec	
24	NC	5X	2 sec	
25	NC	10X	1 sec	
26	NC	10X	2 sec.	

All turned out good!

2/19/96 DISK214

Calculating % Survival with a-Chac + folic acid data from 2/13/96 pg. 83-85.

DAY 1	0mg/L AC	2.5mg/L AC	5mg/L AC	10mg/L AC
0mg/L FA	100%	100%	74% ~~100%~~	0%
10mg/L FA	100%	100%	98%	0%
20mg/L FA	100%	98.7%	92%	0%
30mg/L FA	100%	100%	98.7%	0%
40mg/L FA	100%	100%	100%	0%
50mg/L FA	100%	100%	100%	0%
DAY 2				
0 mg/L FA	96%	60%	0%	0%
10 "	98%	90%	6%	6%
20 "	98%	85.3%	9.3%	0%
30 "	100%	94.7%	6.7%	0%
40 "	96%	97.3%	25.3%	0%
50 "	98%	92%	12%	0%
DAY 3				
0 mg/L FA	96%	44%	0%	0%
10 "	98%	72%	0%	0%
20 "	94%	78.7%	0%	0%
30 "	100%	88%	1.3%	0%
40 "	96%	93.3%	13.3%	0%
50 "	98%	86%	4%	0%
DAY 4				
0 mg/L FA	96%	16%	0%	0%
10 "	98%	66%	0%	0%
20 "	94%	~~74.6%~~ 74.7%	0%	0%
30 "	100%	~~86.6%~~ 86.7%	0%	0%
40 "	94%	78.7%	0%	0%
50 "	96%	84%	0%	0%

Graphs on DISK214 saved as D1AC2_13.IPG, D2AC2_13.IPG, D3..., D4...

COMBO_AC.IPG => 1 page w/ 4 graphs

2/21/96

Tried injecting embryos with 4% fluro-gold, made upon 1/18/96. Used micropipet puller to make microelectrodes. Put on end of Nanoject unit + drew fluro-gold into micropipet. Injected embryos (hopefully) with this setup. One problem we had ~~is~~ was the embryo leaking when the microelectrode was pulled back out. Tried to see embryos fluorescing under the microscope but we had no luck seeing any fluorescend.

Set up more dishes of TMT. Used albino embryos and same setup as on page 85. Used TMT stock (made 2/14/96) 10mg/L. Embryos were laid 2/21/96. In TMT @ 10:30.

2/22/96

Added 40ul di-4 to embryos in dishes of TMT. (9:15 am) - Did not finish.

3/2/96

Modifications to Chicken data - Days 10 - 40. Spreadsheets are saved on DISK 220 along with graphs (in QuattroPro.)

Changes to:

Cardiac 10 - nothing
20 - Di-4 Column 2A/1A+ ÷ 10
30 - Di-4 column 2A/1A+ ÷ 100
40 - Di-4 Column 2A/1A+ ÷ 10

3/2/96 continued

Gastrocnemius 10 - BCECF 2A/1A ÷ 10
20 - Di-4 column 2A/1A+ ÷ 10
30 - Di-4 added 10,500 to 1A and 2A then ÷ column 2A/1A+ by 10
40 - Di-4 column 2A/1A+ ÷ 10

Intestinal 10 - BCECF column 2A/1A ÷ 10
20 - Di-4 column 2A/1A+ ÷ 10
30 - Di-4 added 12,500 to 1A and 2A. then ÷ column 2A/1A+ by 100.
40 - Di-4 column 2A/1A+ ÷ 10

Rectoral 10 - BCECF column 2A/1A ÷ 10
20 - Di-4 column 2A/1A+ ÷ 10
30 - added 13,000 to 1A and 2A then ÷ column 2A/1A+ by 100.
40 - Di-4 column 2A/1A+ ÷ 10

Renal 10 - BCECF column 2A/1A ÷ 10
20 - Di-4 column 2A/1A+ ÷ 10
30 - Di-4 added 12,000 to 1A + 2A then ÷ column 2A/1A+ by 100.
40 - Di-4 column 2A/1A+ by 10.

Sciatic 10 - BCECF column 2A/1A ÷ 10
20 - Di-4 column 2A/1A+ ÷ 10
30 - Di-4 " " ÷ 100
40 - Di-4 " " ÷ 10.

Vagus 10 - nothing
20 - Di-4 column 2A/1A+ ÷ 10
30 - Di-4 " " ÷ 100
40 - Di-4 " " ÷ 10

NOTE** Some di-4's were negative so 10,000 was originally added to all 1A's + 2A's of Di-4, then other modifications were made.

3/4/96 This test we did not like - dumped

FETAX test using high Folic Acid (100mg/L) and α-chaconine (50mg/L stock made 3/4/96) with albino embryos. Used Mendel's 2-9-96 α chac. Page 63 has measurement for FA + AC.

ISH	CONCEN.	DAY0	3/5 DAY1		3/6 DAY2		3/7 DAY3	3/8 DAY4
1	NC	25	23		23		23	23
2	NC	25	24	72	24	72	24	24
3	NC	25	25		25		25	25
4	10mg/L FA, 0 AC	25	24	48	23	47	23	23
5	"	25	24		24		24	24
6	20mg/L FA, 0 AC	25	24	49	24	49	24	24
7	"	25	25		25		25	25
8	30mg/L FA, 0 AC	25	25	49	24 5	48	24	24
9	"	25	24		23		23	23
10	40mg/L FA, 0 AC	25	25	49	25	49	25	25
11	"	25	24		24		24	24
12	50mg/L FA, 0 AC	25	25	50	25	49	25	25
13	"	25	25		24		24	24
14	0 FA, 2.5mg/L AC	25	25	2	1	16	1	1
15	"	25	25		15		14	11
16	10mg/L FA, 2.5mg/L AC	25	25	50	15	21	7	7
17	"	25	25		6		2	2
18	20mg/L FA, 2.5mg/L AC	25	25		0		0	0
19	"	25	25	75	2	6	1	1
20	"	25	25		4		3	3
21	30mg/L FA, 2.5mg/L AC	25	24		11		6	6
22	"	25	25	73	16	29	8	5
23	"	25	24		2		1	1
24	40mg/L FA, 2.5mg/L AC	25	25		10		7	5
25	"	25	25	75	8	24	7	7
26	"	25	25		6		2	2
27	50mg/L FA, 2.5mg/L AC	25	25	50	11	14	6	6
28	"	25	25		3		3	3
29	0 FA, 5mg/L AC	25	22	45	0		0	0
30	"	25	23		0		0	0
31	10 FA, 5mg/L AC	25	24	47	0		0	0
32	"	25	23		0		0	0

3/4/96 cont

	CONCEN.	DAY0	DAY1	DAY2	DAY3	DAY4
33	20mg/L FA, 5mg/L AC	25	25	0	0	0
34	"	25	25	0	0	0
35	"	25	24	0	0	0
36	30mg/L FA, 5mg/L AC	25	25	0	0	0
37	"	25	24	0	0	0
38	"	25	25	0	0	0
39	40mg/L FA, 5mg/L AC	25	25	0	0	0
40	"	25	25	0	0	0
41	"	25	25	0	0	0
42	50mg/L FA, 5mg/L AC	25	24	1	1	1
43	"	25	25	0	0	0
44	0 FA, 10mg/L AC	25	0	0	0	0
45	"	25	0	0	0	0
46	10mg/L FA, 10mg/L AC	25	0	0	0	0
47	"	25	0	0	0	0
48	20mg/L FA, 10mg/L AC	25	0	0	0	0
49	"	25	2	0	0	0
50	"	25	0	0	0	0
51	30mg/L FA, 10mg/L AC	25	5	0	0	0
52	"	25	12	0	0	0
53	"	25	0	0	0	0
54	40mg/L FA, 10mg/L AC	25	0	0	0	0
55	"	25	0	0	0	0
56	"	25	3	0	0	0
57	50mg/L FA, 10mg/L AC	25	1	0	0	0
58	"	25	0	0	0	0

↑ 3% formalin

3/5/96 DBK 214

Calculating % Survival with α-Chaconine and folic acid data from 3/4/96. Pg. 90-91.

DAY1	0 mg/L AC	2.5 mg/L AC	5 mg/L AC	10 mg/L AC
0 mg/L FA	96%	100%	90%	0%
10 mg/L FA	96%	100%	94%	0%
20 mg/L FA	98%	100%	98.7%	2.7%
30 mg/L FA	98%	97.3%	98.7%	22.7%
40 mg/L FA	98%	100%	100%	4%
50 mg/L FA	100%	100%	98%	2%
DAY2				
0 mg/L FA	96%	32%	0%	0%
10 "	94%	42%	0%	0%
20 "	98%	8%	0%	0%
30 "	96%	58%	0%	0%
40 "	98%	48%	0%	0%
50 "	96%	28%	2%	0%
DAY3				
0 mg/L FA	96%	30%	0%	0%
10 "	94%	18%	0%	0%
20 "	98%	5.3%	0%	0%
30 "	94%	20%	0%	0%
40 "	96%	21.3%	0%	0%
50 "	98%	18%	2%	0%
DAY4				
0 mg/L FA	96%	24%	0%	0%
10 "	94%	18%	0%	0%
20 "	98%	5.3%	0%	0%
30 "	94%	16%	0%	0%
40 "	98%	18.7%	0%	0%
50 "	98%	18.0%	2%	0%

Spreadsheets saved as D1AC3_4.WQ1 ...
Graphs saved as D1AC3_4.IPG, D2AC3_4.IPG, etc...
Combination graph saved as Combo3_4.IPG

3/5/96

Set up 3 dishes (TMT) for injecting Fluoro-Gold. (NC, 1mg/L TMT, .5mg/L TMT). Put 15 albino embryos (24+hrs old) in each dish. Added 40 µl di-4 to each dish, also.
Set up camera + intensifier. Tried getting embryos in focus. Need to turn intensifier 3 times counterclockwise in order to get embryos in focus.

3/7/96
Set up FETAX experiment with a Chaconine vs. high Folic Acid. (3rd test). a Chac stock (50mg/L) was made 3/4/96. Folic Acid stock (100 mg/L) was made 3/7/96. See page 83 for AC & FA measurements in each dish. Used albino embryos.

Digitized on Disk ~~225~~ saved as ACFA3-7.PRN

DISH	Concent.	3/7 DAY 0	3/8 DAY 1	3/9 DAY 2	3/10 DAY 3	3/11 DAY 4
1	NC	25	24	24	24	24
2	NC	25	25	25	25	25
3	NC	25	25	25	25	25
4	10mg/L FA, OAC	25	25	25	25	25
5	"	25	25	25	25	25
6	20mg/L FA, OAC	25	25	25	25	25
7	"	25	25	25	24	24
8	30mg/L FA, OAC	25	25	25	25	25
9	"	25	25	25	25	25
10	40mg/L FA, OAC	25	25	25	25	25
11	"	25	25	25	25	25
12	50mg/L FA, OAC	25	25	25	25	25
13	"	25	25	25	25	25
14	OFA, 2.5mg/L AC	25	25	25	25	25
15	"	25	25	23	22	20
16	10mg/L FA, 2.5mg/L AC	25	25	25	24	24
17	"	25	25	21	21	21
18	20mg/L FA, 2.5mg/L AC	25	25	24	24	24
19	"	25	25	25	23	23
20	"	25	25	25	23	23

3/7/96 Con't

ISH	CONCEN.	DAY0	DAY1	DAY2	DAY3	DAY4
21	30mg/LFA, 2.5mg/LAC	25	25	24	24	24
22	"	25	25	25	24	24
23	"	25	25	24	24	24
24	40mg/LFA, 2.5mg/LAC	25	24	24	24	24
25	"	25	25	25	25	25
26	"	25	25	25	25	25
27	50mg/LFA, 2.5mg/LAC	25	25	25	25	25
28	"	25	25	25	25	25
29	0 FA, 5mg/LAC	25	16	5	1	0
30	"	25	17	3	1	1
31	10mg/LAC, 5mg/LAC	25	24	10	5	1
32	"	25	24	17	9	0
33	20mg/LFA, 5mg/LAC	25	24	1	0	0
34	"	25	25	6	3	2
35	"	25	24	13	10	8
36	30mg/LFA, 5mg/LAC	25	25	11	8	6
37	"	25	25	12	10	5
38	"	25	25	18	14	14
39	40mg/LFA, 5mg/LAC	25	25	11	5	3
40	"	25	25	12	11	10
41	"	25	25	13	9	8
42	50mg/LFA, 5mg/LAC	25	25	16	10	7
43	"	25	25	7	2	2
44	0 FA, 10mg/LAC	25	0	0	0	0
45	"	25	0	0	0	0
46	10mg/LFA, 10mg/LAC	25	0	0	0	0
47	"	25	0	0	0	0
48	20mg/LFA, 10mg/LAC	25	0	0	0	0
49	"	25	0	0	0	0
50	"	25	0	0	0	0
51	30mg/LFA, 10mg/LAC	25	0	0	0	0
52	"	25	0	0	0	0
53	"	25	0	0	0	0
54	40mg/LFA, 10mg/LAC	25	0	0	0	0
55	"	25	1	0	0	0
56	"	25	0	0	0	0
57	50mg/LFA, 10mg/LAC	25	0	0	0	0
58	"	25	0	0	0	0

Spreadsheets saved as D1AC3_7.wq1, D2AC3_7.wq1, etc...

3/8/96 DISK 214

Calculating % Survival with a Chaconine + Folic Acid data from 3/7/96. Pg 93-94. (3rd run)

DAY 1	0 mg/L AC	2.5 mg/L AC	5 mg/L AC	10 mg/L AC
0 mg/L FA	98.7%	100%	66%	10%
10 "	100%	100%	96%	0%
20 "	100%	100%	97.3%	0%
30 "	100%	100%	100%	0%
40 "	100%	98.7%	100%	1.3%
50 "	100%	100%	100%	16%
DAY 2				
0 mg/L FA	98.7%	96%	16%	0%
10 "	100%	92%	54%	0%
20 "	100%	98.7%	26.7%	0%
30 "	100%	97.3%	54.7%	0%
40 "	100%	98.7%	48%	0%
50 "	100%	100%	46%	0%
DAY 3				
0 mg/L FA	98.7%	94%	4%	0%
10 "	100%	90%	28%	0%
20 "	96%	93.3%	17.3%	0%
30 "	100%	96%	42.7%	0%
40 "	100%	98.7%	33.3%	0%
50 "	100%	100%	24%	0%
DAY 4				
0 mg/L FA	98.7%	90%	2%	0%
10 "	100%	90%	2%	0%
20 "	98%	93.3%	13.3%	0%
30 "	100%	96%	33.3%	0%
40 "	100%	98.7%	28%	0%
50 "	100%	100%	18%	0%
				0%

Graphs saved as D1AC3_7.IPG, D2AC3_7.IPG, etc...
Combination graph saved as Combo3_7.IPG

3/13/96

Dyed daphnia neonates from today in 50ul di-4 ANEPPS for approx. 2 hours. Will be looking at them in the new fluorescent wells (black + white) from Nunc.

3/14/96 [TEST A] DISK 214

Set up FGTAX experiment w/ a Chaconine vs. high Folic Acid (4th test). Recieved a Chac from Mendel on 3/13/96. a Chac stock (50mg/L) was made 3/14/96. Folic Acid stock (100mg/L) was made 3/14/96. See page 83 for AC + FA measurements in each dish. Used albino embryos.

DISH	CONCENT.	3/14 DAY0	3/15 DAY1	3/16 DAY2	3/17 DAY3	3/18 DAY4
1	NC	25	25	25	22	~~22~~ 21
2	NC	25	25	24	23	22
3	NC	25	25	25	25	23
4	OAC, 10mg/L FA	25	25	25	24	24
5	"	25	25	24	23	20
6	OAC, 20mg/L FA	25	24	24	24	24
7	"	25	25	25	23	21
8	OAC, 30mg/L FA	25	25	22	21	20
9	"	25	25	23	23	23
10	OAC, 40mg/L FA	25	25	24	24	24
11	"	25	25	24	22	20
12	OAC, 50mg/L FA	25	25	23	22	21
13	"	25	25	25	25	24
14	2.5mg/L AC, 0 FA	25	25	23	22	20
15	"	25	25	19	10	8
16	2.5 AC, 10 FA	25	25	22	20	16
17	"	25	25	22	13	11
18	2.5 AC, 20 FA	25	25	24	23	22
19	"	25	25	24	24	23
20	"	25	25	21	17	12
21	2.5 AC, 30 FA	25	25	23	22	20
22	"	25	25	21	16	16
23	"	25	25	23	11	11

DISH	CONCENT.	DAY0	DAY1	DAY2	DAY3	DAY4
24	2.5AC, 40FA	25	25	26	23	22
25	"	25	25	22	20	16
26	"	25	25	24	22	21
27	2.5AC, 50FA	25	25	24	23	22
28	"	25	25	25	22	21
29	5AC, 0FA	25	16	0	0	0
30	"	25	13	0	0	0
31	5AC, 10FA	25	24	3	1	0
32	"	25	20	1	0	0
33	5AC, 20FA	25	25	6	3	0
34	"	25	25	6	0	0
35	"	25	25	8	3	0
36	5AC, 30FA	25	25	6	0	0
37	"	25	23	11	4	0
38	"	25	25	13	7	1
39	5AC, 40FA	25	24	3	0	0
40	"	25	25	7	7	3
41	"	25	24	9	3	2
42	5AC, 50FA	25	25	11	7	4
43	"	25	25	5	1	0
44	10AC, 0FA	25	0	0	0	0
45	"	25	0	0	0	0
46	10AC, 10FA	25	0	0	0	0
47	"	25	0	0	0	0
48	10AC, 20FA	25	0	0	0	0
49	"	25	0	0	0	0
50	"	25	0	0	0	0
51	10AC, 30FA	25	0	0	0	0
52	"	25	0	0	0	0
53	"	25	0	0	0	0
54	10AC, 40FA	25	0	0	0	0
55	"	25	0	0	0	0
56	"	25	0	0	0	0
57	10AC, 50FA	25	0	0	0	0
58	"	25	2	0	0	0

↑ 3% formalin

Spreadsheets saved on Disk 214 as D1AC3_14.WQ1, D2AC3_14.WQ1, etc...
Digitized on Disk 225 saved as ACFA3_14.PRN

3/15/96 Disk 214B [TEST A]

Calculating % Survival w/ 2 Chac + Folic Acid data from 3/14/96, Pg 96-97 (4th run)

DAY 1	0mg/LAC	2.5mg/LAC	5mg/LAC	10mg/LAC
0mg/LFA	100%	100%	58%	0%
10 "	100%	100%	88%	0%
20 "	98%	100%	100%	0%
30 "	100%	100%	97.3%	0%
40 "	100%	100%	97.3%	0%
50 "	100%	100%	100%	4%
		~~97.3%~~		
DAY 2				
0mg/LFA	98.7%	84%	0%	0%
10 "	98%	88%	8%	0%
20 "	98%	92%	26.7%	0%
30 "	90%	89.3%	~~60%~~ 40%	0%
40 "	96%	94.7%	25.3%	0%
50 "	96%	98%	32%	0%
DAY 3				
0mg/LFA	93.3%	64%	0%	0%
10 "	94%	66%	2%	0%
20 "	94%	85.3%	8%	0%
30 "	88%	69.3%	14.7%	0%
40 "	92%	86.7%	13.3%	0%
50 "	94%	90%	16%	0%
DAY 4				
0mg/LFA	89.3%	56%	0%	0%
10 "	86%	54%	02%	0%
20 "	90%	76%	0%	0%
30 "	86%	66.7%	1.3%	0%
40 "	88%	78.7%	6.7%	0%
50 "	90%	86%	8%	0%

Graphs on Disk 214B saved as: D1AC314A.IPG, D2AC314A.IPG, etc...

3/14/96 [TEST B] DISK 214

Set up a 2nd FETAX experiment. Same exp. as TEST A. Same stock solutions, same embryos. Set up @ 4:00 pm

		3/14	3/15	3/16	3/17	3/18
	CONCEN. (mg/L)	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4
1	NC	25	25	23	23	23
2	NC	25	25	25	24	23
3	NC	25	25	25	24	23
4	OAC, 10 FA	25	25	25	24	23
5	"	25	25	24	24	24
6	OAC, 20 FA	25	25	24	22	22
7	"	25	25	25	24	21
8	OAC, 30 FA	25	25	25	~~24~~ 25	25
9	"	25	25	25	25	25
10	OAC, 40 FA	25	25	25	25	24
11	"	25	25	24	24	24
12	OAC, 50 FA	25	25	24 ~~24~~	23	23
13	"	25	25	25 ~~20~~	22	21
14	2.5 AC, 0 FA	25	25	~~20~~ 17	7	7
15	"	25	25	17 ~~20~~	12	12
16	2.5 AC, 10 FA	25	25	~~20~~ 19	10	7
17	"	25	25	19	15	14
18	2.5 AC, 20 FA	25	25	25	17	16
19	"	25	25	21	19	17
20	"	25	25	22	13	10
21	2.5 AC, 30 FA	25	25	23	15	14
22	"	25	25	24	21	20
23	"	25	25	23	15	12
24	2.5 AC, 40 FA	25	25	25	25	25
25	"	25	25	25	17	13
26	"	25	25	24	20	16
27	2.5 AC, 50 FA	25	25	20	19	18
28	"	25	25	24	22	19
29	5 AC, 0 FA	25	24	2	0	0
30	"	25	23	0	0	0
31	5 AC, 10 FA	25	24	1	0	0
32	"	25	24	4	0	0
~~33~~		~~25~~				
~~34~~		~~25~~				

	CONCEN	DAY0	DAY1	DAY2	DAY3	DAY4
1	5AC, 20FA	25	25	5	3	3
2	"	25	25	4	2	2
3	"	25	25	1	0	0
4	5AC, 30FA	25	25	7	23	2
5	"	25	25	9	3	0
6	"	25	25	9	0	0
7	5AC, 40FA	25	25	5	4	1
8	"	25	25	10	3	3
9	"	25	25	4	0	0
10	5AC, 50FA	25	25	6	3	1
11	"	25	25	2	1	0
12	10AC, 0FA	25	0	0	0	0
13	"	25	0	0	0	0
14	10AC, 10FA	25	0	0	0	0
15	"	25	0	0	0	0
16	10AC, 20FA	25	0	0	0	0
17	"	25	0	0	0	0
18	10AC, 30FA	25	0	0	0	0
19	"	25	0	0	0	0
20	10AC, 40FA	25	0	0	0	0
21	"	25	0	0	0	0
22	10AC, 50FA	25	0	0	0	0
23	"	25	0	0	0	0

Digitized on Disk 225 saved as ACFA314B.PRN

↑ 3% formalin

Spreadsheets saved on Disk 24 saved as:
D1AC314B.WQ1, D2AC314B.WQ1, D3AC314B.WQ1,
D4AC314B.WQ1